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TUBERCULOUS PERICARDITIS WITH EFFUSION: THE IMPACT OF ANTIMICROBIAL THERAPY

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WITH the advent of streptomycin and other more recent antituberculous drugs, the entire outlook in the treatment of tuberculosis has changed. This is well illustrated by the mortality trend between 1943 and 1951 on the tuberculosis wards of the Philadelphia General Hospital. Figure 1 shows that the number of deaths per year remained rather constant at close to 500 until 1948. At this time the impact of streptomycin was felt and the number of deaths each year has rapidly declined, in spite of the fact that the average daily census increased from about 250 to about 375. In addition the total disposition of patients (discharges plus deaths) has decreased, so that the average duration of hospitalization has more than tripled. This phenomenon is certainly not limited to one institution and it is likewise not limited to pulmonary tuberculosis but has been noted in all forms of the disease.

Much has been written about the influence of streptomycin and other antimicrobials on pulmonary tuberculosis. Because tuberculous pericarditis is relatively uncommon, little has appeared in the literature concerning the impact of these drugs on this form of the disease. Since the value of streptomycin has been well established in the treatment of tuberculosis, a scientifically controlled investigation of its effect on an uncommon disease such as tuberculous pericarditis is not now justified. Therefore we must be satisfied with a comparison of treated and untreated cases at the same institution with the hope that the comparison is not invalidated by unrecognized factors.

This paper has been written in an attempt to point out what differences exist in the short-term prognosis of tuberculous pericarditis since the introduction of antituberculous agents through a comparison of treated cases with an untreated series drawn from this institution.

Material. The records of 34 cases of tuberculous pericarditis with effusion admitted to the Philadelphia General Hospital from 1941 to 1952 form the basis of this investigation. Twenty patients did not receive antimicrobial therapy for tuberculosis and 14 patients did. Both groups have been divided into proven cases and presumptive cases. Proven cases were those in which tubercle bacilli were isolated from the pericardial fluid or in which the clinical diagnosis of tuberculous pericarditis was confirmed by necropsy. The presumptive cases were those in which there was extrapericardial bacteriologic or histologic evidence of active tuberculosis in conjunction with pericardial effusion. The diagnostic criteria for the presence of tuberculous pericarditis included evidence obtained by physical examination, roentgenography, electrocardiography, bacteriologic and histologic studies.

The course of the disease was evaluated by temperature changes, serial electrocardiograms and roentgenograms whenever available, and by follow-up after discharge in most cases. The antimicrobial therapy was studied with respect to method of administration, dosage and duration. Only a few cases were personally observed by the authors. Information in the majority was obtained from the hospital records.

The background data for the 20 untreated cases (those that did not receive antimicrobial drugs) and the 14 treated cases (those that did receive antimicrobial drugs) are illustrated in Figs. 2 to 5. Figure 2 shows that the percentage of proven cases was the same in both groups of patients. It was noted that all deaths in the treated series occurred in proven cases; however, these are weighted with 2 cases that required necropsy to confirm the clinical diagnosis of tuberculous pericarditis. It was also noted that the only

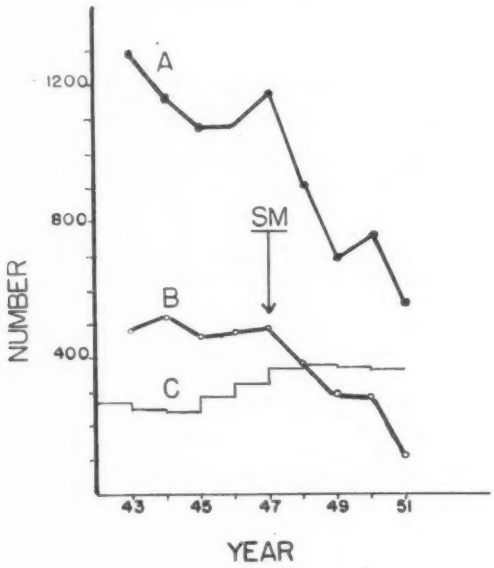

FIG. 1—Disposition of patients with tuberculosis in the Department of Chronic Diseases of the Chest, Philadelphia General Hospital, from 1943 to 1951. A is the total disposition of patients, including both deaths and discharges. B is the number of deaths. C is the average daily census. SM indicates the approximate introduction of streptomycin. Note that there was a definite fall in deaths and total disposition of patients following the introduction of streptomycin, despite the increase in average daily census. The number of deaths fell a little more rapidly than the total disposition. The fall in total disposition is accompanied by an increase in the average duration of hospitalization.

patients in the untreated series who survived were presumptive cases. Again the proven cases were weighted with 4 cases that required necropsy to confirm the clinical diagnosis of tuberculous pericarditis.

All but one of the untreated patients and all of the treated ones were Negroes. The proportion of males to females was somewhat higher among the treated cases.

graver prognosis according to Wood[22]. We are unable to confirm this, since 1 of 6 patients over the age of 50 and 4 of 28 patients under 50 are asymptomatic after discharge.

Figure 2 shows that there was no significant difference between treated and untreated patients with respect to the proportions showing clinical evidence of pericariditis, whether by physical examination, roentgenogram or

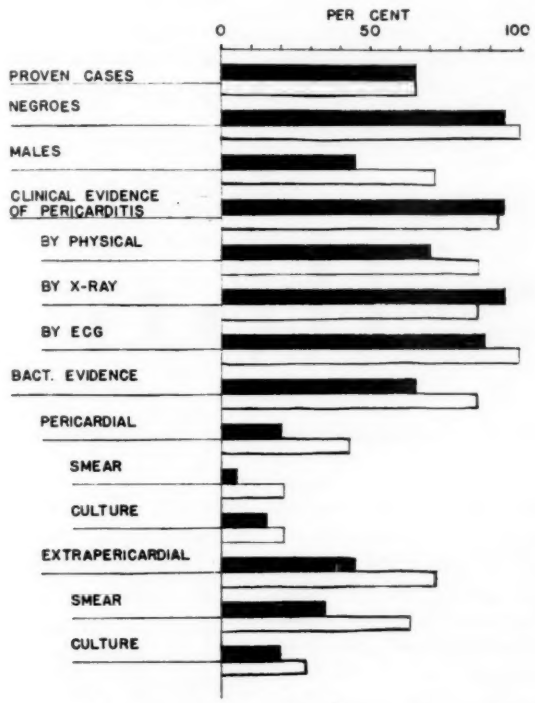

FIG. 2.—Comparison of untreated cases (black bars) with treated cases (white bars) with respect to certain background data.

The age distribution is shown in Fig. 3 and it does not differ very much in the 2 groups. The average age was 34 years in the untreated cases and 32 years in the treated group. In the entire series 71% were less than 40 years old. However, there are a few more patients in the untreated group over the age of 50 years and this carries a

electrocardiogram. All but one case in each group showed some clinical evidence of pericardial effusion. One of the two exceptions was suspected of having an effusion on fluoroscopy. Pericarditis with effusion was confirmed in both cases at necropsy and 200 cc. of amber fluid were found in the pericardial sac in each case. Prob-

ably effusions of less than 300 cc. are missed on physical examination[21]. A presumptive diagnosis of pericardial effusion is possible by roentgenogram when the amount of fluid exceeds 200 cc.[18].

The treated cases in general were more often positive for tubercle bacilli on smear or culture of material from the pericardial sac or from extrapericardial sources (Fig. 2). This is probably explained by the fact that almost all of the untreated patients were admitted during the pre-streptomycin era when less use was made of the tuberculosis bacteriologic laboratory at this institution. It was necessary to depend on a positive smear for acid-fast bacilli as the sole means of diagnosis in only one case in each group. In all other instances the diagnosis of tuberculosis was established by positive culture or histologic examination.

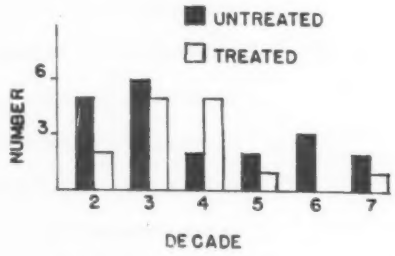

FIG. 3.—Age distribution of treated and untreated cases.

Figure 4 shows that the proportion of patients in each group with histopathologic evidence of tuberculosis was approximately the same. The percentage of necropsies was somewhat higher in the untreated series because the percentage of deaths was higher. However, the percentage of cases in which necropsy was required to confirm the clinical impression of tuberculous pericarditis was almost the same in each group. The percentage of cases developing pericarditis while in the hospital was almost the same in both treated and untreated patients and the same was true of the most important tuberculous complications with the exception of peritonitis. Tuberculous peritonitis was found more often in the untreated cases than among the treated patients, and this tends to worsen the prognosis of the former group. The explanation probably lies in the fact that there was a higher proportion of necropsies in the untreated cases and some instances of peritonitis among the treated cases may not have been recognized clinically, or may have been prevented by the antimicrobial therapy. However, this worsening of prognosis in the untreated series is probably counterbalanced by the fact that there were among the treated cases two patients who died of empyema, one who died of tuberculous meningitis and one who died of a spontaneous pneumothorax. It is important to note that, among the treated cases, of the 6 patients who died, 2 had empyema and 4 had miliary tuberculosis (including 1 of the empyema cases). Both of these complications, of course, carry a very serious prognosis, even in these days of antimicrobials. In contrast, of the 8 treated patients who survived, only 2 had miliary tuberculosis; 1 is still under therapy and the other has had only a 5-

month follow-up after discharge so that the eventual outcome is uncertain.

Non-tuberculous complications that seem to be important are cardiac failure and cardiac arrhythmias. These complications have an adverse effect on prognosis. Of 8 patients discharged alive, none had an arrhythmia and only 3 (38%) had cardiac failure. In contrast, of 24 patients who died, 9 (37%) had arrhythmias and 17 (71%) had cardiac failure. However, both of these complications were evenly distributed between the untreated and treated groups, since 60% of the untreated patients and 64% of the treated patients had cardiac failure, while 30% of the untreated cases and 36% of the treated cases had arrhythmias.

Figure 5 shows that the duration of symptoms prior to admission to the hospital was the same in both treated and untreated patients. In view of what has been said above, we can conclude that *both treated and untreated patients in this series were similar with regard to various measurable characteristics and therefore the prognosis was essentially the same in both groups.*

Results. Having shown that these 2 groups of patients are fairly comparable, we may now look at the difference in therapeutic results as summarized in Fig. 6. Fever improved in the majority of treated cases but did not improve in most of the untreated cases. Figure 7 shows this with respect to time. The few untreated patients whose fever did improve showed the improvement within one month of admission. Among the treated patients, most of the patients who improved also did so within one month of admission, but 3 patients required from 2 to 6 months for their temperature to reach normal. The range was 2 to 173 days with an average of 46 days.

There was also a significant difference (Fig. 6) between the untreated

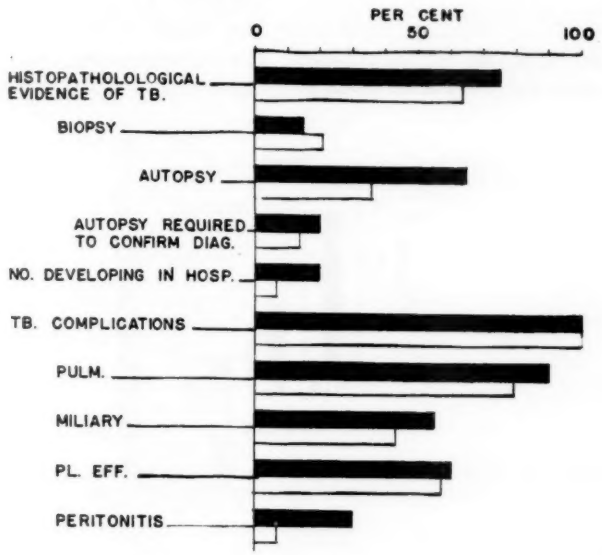

FIG. 4.—Comparison of untreated cases (black bars) with treated cases (white bars) with respect to certain background data.

and treated patients with respect to reduction of cardiac size on roentgenogram where serial films were available (25% of 12 untreated cases and 75% of 12 treated cases) but there was relatively little difference with respect to return of the electrocardiogram to normal where serial tracings were available (11% of 9 untreated cases and 30% of 10 treated cases). When electrocardiographic improvement occurred, it was slow.

The antimicrobial therapy used included streptomycin in all 14 treated patients. In addition *para*-aminosalicylic acid was used in 8 patients and isoniazid in 2. The method of administration varied considerably over the years since streptomycin has been available. It was given continuously (daily) in half of the patients and 5 of the 7 died. However, the deaths can be better related to the duration of therapy than to the method of administration (see below). Intermittent streptomycin was used in the other 7 patients (1 gm. intramuscularly several times a week) but in 2 of these the streptomycin was given continuously for a short time first and in 4

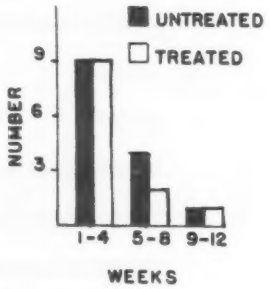

FIG. 5.—Comparison of treated and untreated cases with respect to the number of weeks symptoms existed prior to hospitalization.

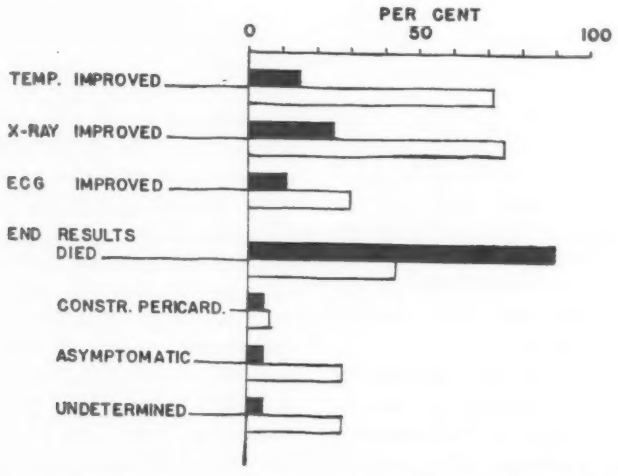

FIG. 6.—Comparison of untreated cases (black bars) and treated cases (white bars) with respect to results.

patients the streptomycin was given in 2 or more courses interrupted by weeks or months. The Fitzsimons regimen for miliary-meningeal tuberculosis was used in one case[16]. Of the 8 patients who were treated with PAS, 3 patients have died (although one was treated for only one day) and one has had no follow-up, as he can not be traced. We can not draw any conclusions regarding the efficacy of PAS when added to streptomycin in all are dead. It should be noted that 2 of these 4 patients died within less than 50 days and therefore did not have the opportunity to receive more than 50 days of treatment.

The end-results are shown in Fig. 6. The mortality rate has been cut in half by drug therapy: from 90% to 43%. Constrictive pericarditis was found in only one case in each group. In the untreated group, only one patient is known to be asymptomatic 16 months

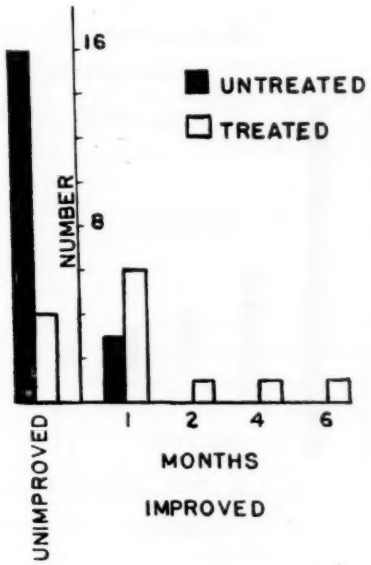

FIG. 7.—Temperature response in untreated and treated cases.

this small series. The 2 patients who received isoniazid are still under therapy.

The duration of therapy seems to be of some importance with respect to the results. Ten of the 14 patients received antimicrobials for more than 50 days, and only 2 of these died. Both of the latter had tuberculous empyema (pleural), a complication which even with antimicrobial therapy carries a grave prognosis. Of the 4 patients who received less than 50 days of therapy, after discharge, whereas in the treated group 4 patients are known to be asymptomatic up to 3 years after discharge. In the untreated group no follow-up information was obtained for one of 2 patients discharged alive, and in the treated group 2 patients are still under therapy while no follow-up information was available for 2 other cases after discharge. In order to avoid giving the benefit of the doubt to the treated cases, we have classified those patients as dead for whom we have

been unable to obtain follow-up information for the purposes of Fig. 8. In this chart is depicted the duration of the disease from the onset to death or the present date of follow-up (September, 1952). It will be noted that 19 of the 20 untreated cases are dead (including one patient discharged alive with no follow-up) and that most of them died within 4 months (average 3.3 months) of the onset of tuberculous pericarditis. Of the 14 treated cases, 8 are dead (including 2 patients discharged alive with no follow-up) and most of these also died within 4 months of the onset. Two patients are still under therapy and 4 patients are well. Three of these have had a longer asymptomatic follow-up course than the single untreated patient who survived. The follow-up period after discharge in these 4 cases varied from 5 to 36 months. The patient with the shortest follow-up period had miliary tuberculosis and was treated with the Fitzsimons regimen[16] while in the hospital. Since discharge he has been receiving 1 gm. of streptomycin intramuscularly once a week (5 months).

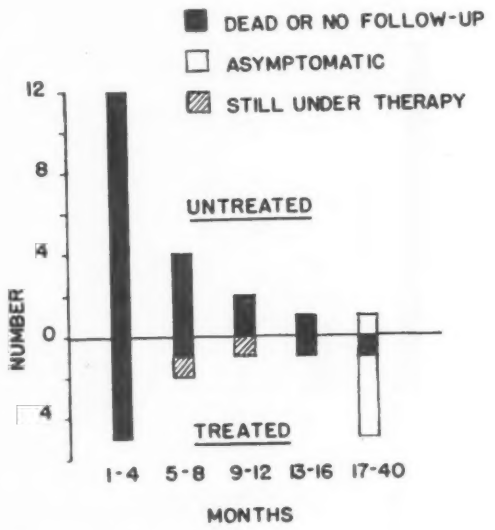

FIG. 8.—Length of observation of treated and untreated cases until death (discharge if no follow-up) or present status.

Discussion. Tuberculous pericarditis is uncommon[8], even in large institutions for the treatment of tuberculosis[6]. This accounts for the paucity of literature on the antimicrobial therapy of this lesion[4,5,10,12-15,20]. Nevertheless tuberculosis is probably second only to rheumatic fever as a cause of pericarditis with effusion[3,19]. Therefore an effective treatment is important. The difficulties in evaluating the efficacy of a new therapy for an uncommon disease are obvious.

However, if a new treatment is really effective, it is not too difficult to demonstrate its value in a rough way, even in the absence of a controlled investigation, by comparison with the results obtained in the past at the same institution if those results have been generally bad. This approach has been used justifiably in evaluating the benefits of various

drugs in treating miliary and meningeal tuberculosis, because the fatality rate without modern therapy is known to be practically 100%. We have used the same approach to evaluate the use of antimicrobials for tuberculous pericarditis, because the outlook for this disease without these drugs is known to be generally poor.

The mortality rate of untreated tuberculous pericarditis in proven cases is uniformly high[3,6-8,11,22] and comparable with the rate of 90% in our untreated cases. The mortality of this disease may not actually be so high when one considers that reports on proven cases may be weighted with cases that require necropsy confirmation of the clinical diagnosis, and many cases not confirmed by necropsy are excluded. However, the error involved may be greater if one includes unproven cases, particularly if one includes those cases in which the diagnosis of tuberculosis, whether pericardial or extrapericardial, has not been established.

The duration of the disease from onset to death found in the literature[8,9,11] is also consistent with the average of 3.3 months in our untreated group. It is therefore apparent that tuberculous pericarditis naturally has a very poor outlook.

The prognosis of this disease is dependent on various factors. Age of the patient is one of these. There is some difference of opinion concerning the usual age at which tuberculous pericarditis occurs. Keefer[11] and Blalock and Levy[3] state that most of their patients are over the age of 40 years. Bellet *et al.*[2] in an earlier report from the Philadelphia General Hospital state that the disease is more common in younger patients and this is consistent with other reports in the literature[5,6,9]. Our patients for the most part fell into the younger age groups. The prognosis is said to be adversely affected if the patient is over the age of 50 years[8,22], probably because the aged cardiovascular system is unable to handle the added burden. This does not seem to be true in our series.

Race is another factor. The majority of patients are Negroes and the prognosis of tuberculosis in general is worse in Negroes than in whites. Since all but one of our patients were Negroes, the prognosis in the present series is adversely affected. Sex is probably not a factor in prognosis, although almost all authors are agreed that the disease is more common in males (see below).

Wood[22] as well as Harvey and Whitehill[8] have claimed that the prognosis is worse in proven cases. This is true also in our series, since in the entire group 19 of the 22 proven cases are known to be dead, whereas only 5 of 12 presumptive cases are known to be dead. However, the proportion of proven cases is the same in our treated series as in the untreated series, so that this factor does not invalidate the comparison of our 2 groups of patients.

The most important prognostic factor is the presence of tuberculous complications[5]. We have not confined our cases to so-called clinically primary tuberculous pericarditis, and consequently the prognosis in many of our cases is determined primarily by the presence of serious tuberculosis elsewhere in the body. All of our patients had extrapericardial tuberculous disease, most frequently pulmonary. There was also a high incidence of miliary and serosal forms of tuberculosis. Tuberculous pericarditis itself is frequently an expression of hematogenous dissemination of the disease, and this probably accounts in great measure for its high fatality rate. The frequent association of tuberculous pericarditis with other serosal forms of tuberculosis has been demonstrated by Auerbach in a large series[1].

Wood[22] states that a gradual onset of symptoms carries a grave prognosis, and this seems to be true in our series. The duration of symptoms prior to hospitalization in all cases known to be alive was 4 weeks or less, whereas it varied up to 12 weeks in those known to be dead. However, this factor has the same magnitude in both treated and untreated cases in our series. Wood further states that the occurrence of cardiac arrhythmias makes for a grave prognosis. This has been corroborated in our series along with the adverse effect of cardiac failure, but these factors had the same magnitude in the treated and untreated groups.

We have discussed the above prognostic factors at some length because it is important for us to show that their influence is equal in both groups of the present series, in order to evaluate the effect of antimicrobial therapy. We believe that Figs. 2 to 5 show our treated cases to be quite comparable with our untreated cases with respect to these factors and other measurable characteristics, except that the male sex and bacteriologic evidence of tuberculosis were more common in the treated cases than in the untreated cases. We are unable to show that the latter characteristics materially affect the prognosis. The sex distribution had no influence on mortality because in the entire series, 10 of 19 males died, and 8 of 15 females died. Likewise, the finding of tubercle bacilli in the pericardial fluid had no influence on the mortality rate because 7 (70%) of 10 patients with positive pericardial fluid died, and 17 (74%) of 24 patients with negative pericardial fluid died. The higher incidence of tuberculous peritonitis in the untreated group with its serious prognosis is offset by other serious complications in the treated group. Therefore the 2 groups of patients seem to be generally similar with regard to prognosis. Having established this, we can proceed to evaluate the results obtained.

There is no doubt that the prognosis of tuberculous pericarditis has been altered by antimicrobial therapy, since the mortality rate has been cut from 90% to 43% in our series. This difference is statistically significant according to the method of Peckham[17]. If we consider as dead those patients discharged alive for whom there is no follow-up, the mortality rate has dropped from 95% to 57% in this series. The mortality rate of treated cases may prove to be a little higher than 57% since 2 of our cases are still under therapy and no definite statement can be made on their outcome at this time. The literature on the treatment of this disease with antimicrobials[4,5,10,12-15,20] can not be similarly evaluated, either because the number of treated cases is too small in most of the reports or because no comparison is made with untreated cases at the same institution. The only other relatively large series of treated cases in this country was reported by Falk and Ebert[5], who accumulated 17 cases from the various Veterans Administration Hospitals. Six of their patients have died, thus giving a mortality rate (35%) similar to the rate reported here. Their patients were restricted to those with circulatory failure and their series is therefore not quite comparable with ours.

Among those patients who died we attempted to determine the influence of antimicrobial therapy on the duration of life. Unfortunately the number of cases is too small to allow us to draw any conclusions. Among our untreated cases, 55% of the deaths occurred within 3 months. Among 6 treated patients who died, 3 (50%) died within 3 months. This suggests that, although the mortality rate is lowered by antimicrobial therapy, among those pa-

tients who are destined to die, one-half will die within 3 months whether the patients receive antimicrobials or not.

The response of this disease to drug therapy is usually slow, but occasional cases will show a dramatically rapid improvement as illustrated in a case report by Weiss and Bogen[20]. When the response is rapid, it is limited to the improvement in symptoms and fever. The roentgenographic signs of effusion and the electrocardiographic signs of pericarditis even in these cases improve more slowly.

The drugs and dosages used, both in the literature and in our own series of cases, have varied so greatly that no definite conclusions can be reached regarding the optimum regimen. In the case reported by Weiss and Bogen[20], cure of the pericarditis was obtained with as little as 2 gm. of streptomycin once a week for 6 weeks with daily PAS during the first 12 days. A study of the various regimens used in our cases brings to light perhaps only one important point. Although the number of cases is small, the data suggest that the results are better if therapy is given for more than 50 days. Intrapericardial instillations of streptomycin are seldom used[4,5].

The longest period of observation of cases treated with antimicrobials in the literature is 31 months[14] and the longest period in our series is 40 months. No conclusions can be drawn concerning the long-term results of antimicrobial therapy, but certainly some patients can survive as long as 40 months without recurrence of pericarditis or the development of constrictive pericarditis. No one can predict, however, in view of the fact that pericarditis is often a manifestation of hematogenous tuberculosis, that a patient who recovers from tuberculous pericarditis will not at some time in the future develop a recurrence of tuberculosis.

Summary and Conclusions. A series of 34 cases of tuberculous pericarditis admitted to the Philadelphia General Hospital from 1941 to 1952 has been presented. These were divided into a group of 20 patients who did not receive antimicrobial therapy and a group of 14 patients who did. These 2 groups were comparable with respect to various measurable characteristics. Streptomycin and possibly other drugs such as PAS and isoniazid, produce a definite reduction in the mortality rate and prolongation of life, but, at present no conclusions are warranted concerning the optimal regimen of therapy or the long-term results.

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CORONARY DISEASE IN THE SECOND CENTURY OF LIFE*

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MOST authors describe coronary atherosclerosis as a degenerative condition occurring in middle and old age. They describe the disease as being most common between the ages of 50 and 70 and they suggest that the disease progresses with age. In a very recent article, Medalia and White[1] reported an analysis of 1,251 autopsy protocols of patients of 50 to 90. They observed that the number of patients of both sexes with coronary sclerosis increased with advancing age from the sixth to the ninth decade. However, they also observed that the number of patients with only minimal sclerosis rose sharply in the ninth decade as compared with the other three. They reported no observations on the tenth decade. Therefore, I feel justified in describing 9 patients who have passed the century mark and in displaying their electrocardiograms.

The case of the first patient is described in some detail. He was first seen in 1921, complaining of puffiness of the ankles and some dizziness. He was put on digitalis and his symptoms improved. He was a lawyer of means and enjoyed an excellent cook, elaborate cooking and cigars. His use of alcohol was most moderate; but he was not an abstainer. His immediate family lived into the middle eighties, but not beyond. The first electrocardiogram (Fig. 1A) was made in 1934. At this time he was working every day and, at 84 years of age he was commuting some 25 miles to his office. The next record (B) was made in 1946 as part of a routine check-up. Although he had achieved his 96th birthday and had been paid the face value of his life insurance, he continued to visit his office every day. Some of his money was invested in real estate. The first of each month he was well enough to collect his rents in person. The third record (C) was taken shortly after his hundredth birthday and nearly 30 years after he first complained of edema. He was still enjoying life and was still able to visit his club and play poker with his friends.

Then 4 months later, the patient suffered a severe attack of precordial pain, which radiated into his left arm. His systolic pressure, which for 30 years had varied from 120 to 150, fell to 80. He developed orthopnea, basal rales and fever. The next tracing (D) was taken after the onset of pain. The following day he was admitted to the hospital and for several weeks he was desperately ill. No hope was entertained for his recovery and no anticoagulant therapy was attempted. For several days his blood pressure was unobtainable and his end seemed imminent. However, despite the prognostications of his physicians, he recovered.

Tracing (E), showing partial A-V block and low voltage, was taken during his hospital stay. By the time the final tracing (F), which shows a healed anterior wall infarct, was made, the old man was downstairs for his meals and was being taken for long drives each afternoon.

Nine months later, the subject took to his bed, coughed up some bloody sputum and died in a few days. In addition to pneumonia, his autopsy report read as follows: "The heart is considerably enlarged, particularly in the vertical and transverse diameters. The lower part, about the apical portion, appears distinctly protruded. The major part of the heart supplied by the anterior descending branch forms a huge aneurysm, the walls of which are formed by the lower anterior half of the interventricular septum and of the anterior wall. The wall of this typical Rokitansky aneurysm is unusually thin, almost translucent. Along the endocardial lining of this aneurysm, the diameter of which measures 8 cm., there is seen in parts of the wall, a firmly adherent thrombus. About the entire anterior wall there are several firm adhesions of the pericardium extending also to the lower portion of the posterial wall of the heart. The unusual, and almost complete, dis-

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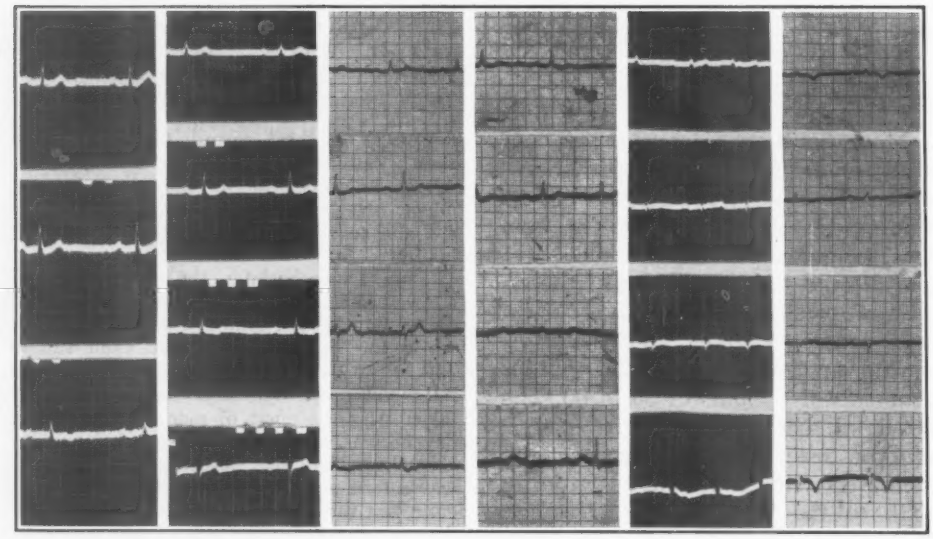

FIG. 1.—(A-F) Tracings before and after coronary occlusion occurring at the age of 100 and with survival of the patient (see text).

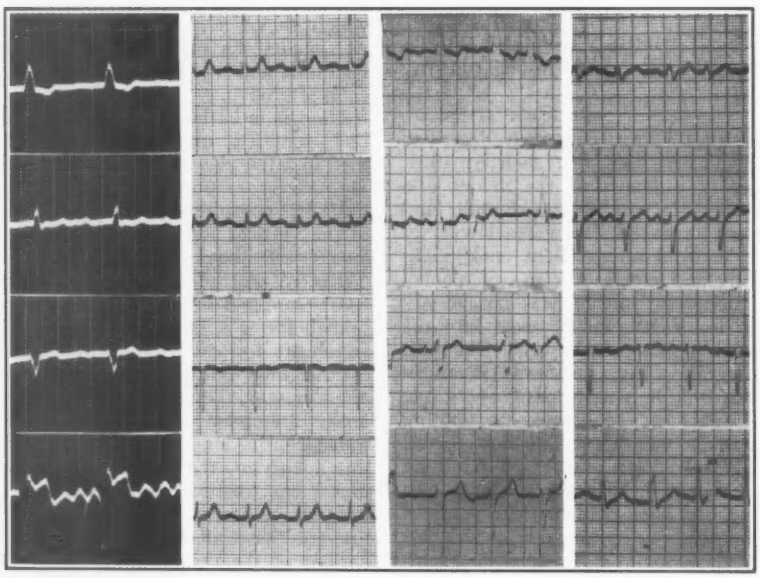

FIG. 2.—(G-J) Tracings of 4 centenarians without symptoms (see text).

appearance of the musculature in the heart wall in and about the area of the aneurysm with the major part of the myocardium of normal thickness, if not slightly hypertrophic, especially close to the mitral valve, is responsible for the somewhat bizarre and elongated shape of this hypertrophic heart."

The next 8 patients will be described very briefly. While most of them had multiple-lead electrocardiograms, for the sake of uniformity, there are shown only the conventional leads and one chest lead.

substitute eggs for meat at a great many meals.

The youngest patient in this series is a Catholic Sister 100.5 years old. Despite her years she has retained her faculties and enjoys reasonably good health. It is now over 80 years since she entered the convent and I suspect that her cholesterol intake has been somewhat restricted. Her electrocardiogram (H) taken a few months ago, can scarcely be regarded as abnormal.

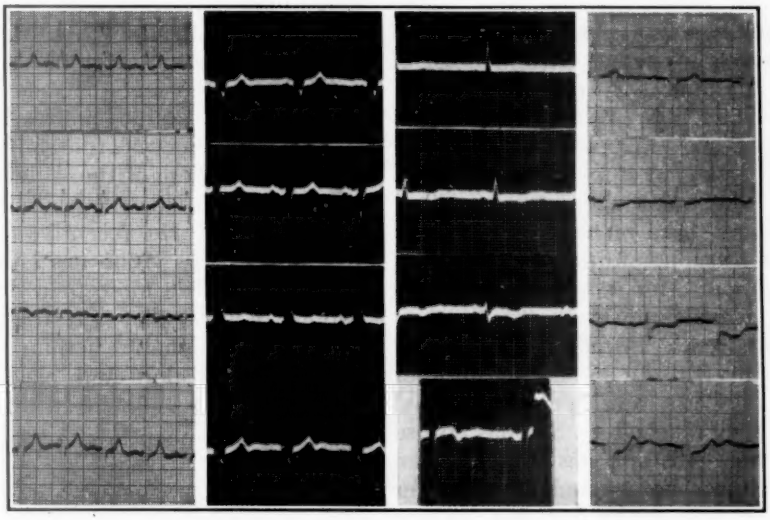

FIG. 3.—(K-N) Tracings of 4 centenarians without symptoms (see text).

The first set of tracings are those of a doctor's grandfather on his hundredth birthday. The widened QRS and prolonged P-R interval suggest the passage of time; but the man never had any cardiac symptoms. He died, a little more than 2 years after this record, of prostatic obstruction and uremia. He was able to be up and about and able to read the paper and follow the news until a few weeks before the end. He ate everything set before him but, during these last years, his dental difficulties caused him to

Tracing (I), which shows a bundle block and ventricular extrasystoles, is that of an inmate of an old folks' home. This woman was brought to this country in a sailing vessel in 1850 and lost contact with her English relatives so that we cannot determine the longevity of the siblings. However, her son, now 82, thinks that they all reached four score years or more. Except for her sight, which failed some 30 years ago, her health has been excellent. She has always avoided milk and cream because she dislikes them; but she has

eaten everything else set before her and has always enjoyed both eggs and butter.

A marked left axis deviation constitutes the only unusual finding in the tracing (J) of a Grand Army Veteran, the last survivor in our part of the state. He was 102 at the time the record was made. At this time he walked to and from his office, a distance of over a mile, and managed his own real estate business. He attributed his health and years to peanut butter. His death at 104 was said to have been caused by pneumonia.

It took two visits into Canada to get this perfectly normal tracing (Fig. 3, K) on a rather spry old lady who is approaching 105. At the time of the first visit she was out calling and I had to make an appointment to find her in. She walks without a cane; but is definitely hard of hearing and there is demonstrable pitting of the ankles after supper. Her most prized possession is a cablegram from the King on her hundredth birthday. While her mother lived to be 95 and one brother died in his 80's, 3 of her siblings died before they reached 40. She has no use for dietary fads and has always eaten as much as she wanted of whatever she likes. She thinks that her favorite food is a soft boiled egg. Since this paper was written, the patient underwent an operation for acute appendicitis. However, she is apparently recovering nicely.

The interventricular conduction defect shown in tracing (L) is justified by the fact that the man, whose heart made it, was 106 years old. He was admitted to the hospital for an acute urinary retention but the jarring of the ambulance was such that he voided spontaneously. He stayed only long enough to have this electrocardiogram and a chest roentgenogram, which was reported as showing the heart not enlarged, the lung field clear and the aorta slightly tortuous. He died, apparently of pneumonia, some months later; but he worked as a cabinet maker until a week before the end.

Electrocardiogram (M) is that of a doctor's mother who will, barring accidents, be 106 next month. She is still able to get to church and is still in possession of her faculties. She has had no dietary restrictions. Her electrocardiogram belies the fact that she finished school before Einthoven was born.

The final electrocardiogram (N) is that of the oldest of the 4 living Civil War veterans. He will be 112 on his next birthday. He recently voted for Eisenhower. His vision has failed until now he has little more than light perception; and his diet is restricted by a huge diaphragmatic hernia; but he seemed quite alert and clear in his mind. A severe bronchiectasis has not impaired his appetite for cigars.

Summary. (1) There are presented data and electrocardiograms of 9 persons aged 100 years or more.

(2) One patient survived a coronary occlusion for 9 months.

(3) If one were to draw conclusions from this small series, they would be: (a) In the making of some human machines a superior grade of tubing is used and (b) tobacco and cholesterol are not always the toxic substances that some believe them to be.

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FOLLOW-UP STUDY OF CLINICAL RESULTS OF ANTI-AMEBIC THERAPY*†

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MANY studies have pointed out that a large proportion of the general population are infested with *Endamoeba histolytica*. Craig[3], for instance, states that at least 10% of the people of the United States as a whole harbor the parasite. He quotes the results of numerous surveys which show that the percentage of the population infested is high even in northern states, for practically nowhere is it under 1%, and it is usually closer to 5%. In spite of this, a diagnosis of disease due to amebic infestation is not commonly made in private or hospital practice particularly in the northern states. To explain this, it has been postulated that many of the patients that carry amebae have no symptoms. Faust[5] stated that 90 to 95% of persons harboring the organisms are apparently symptomless carriers. Others feel that many patients have mild or atypical symptoms that have not been recognized as due to amebic infestation. Craig[3] stated that 65% of individuals passing the cysts of *Endamoeba histolytica* presented some symptoms that might have been due to this parasite. He and others[1,11] indicated that diarrhea may be absent and that constipation, abdominal pain, fever, anorexia, jaundice, muscular aching, weakness and malaise may constitute the clinical evidence of amebiasis. The question arising in a given patient, however, is whether these symptoms, particularly the atypical ones, are due to the amebiasis or whether they are merely found in a patient who happens to be one of the many that harbor ameba.

In this connection we reviewed the records and made follow-up studies of all patients who had been found to have positive stools for *Endamoeba histolytica* while hospitalized at the Cleveland Veterans Administration Hospital from 1947 to 1951. We endeavored to determine which of the symptoms that have been attributed to amebiasis these patients showed, what laboratory evidence for amebiasis they

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presented, and in particular, what follow-up examination had shown to be the ultimate effect, on their symptoms, of treatment for amebiasis.

Methods and Results. Fifty-four patients were found to have amebae in their stool during the years 1947 to 1951. As routine stool examinations are not done in this hospital, each of these cases had been studied because of some element or elements in the clinical picture considered suggestive of amebiasis, such as diarrhea, abdominal pain, fever, jaundice, fistula-in-ano or eosinophilia. We have selected the element in each case that seemed most suggestive of amebiasis and designated this as the primary symptom. Many of these cases had other clinical features seen in amebiasis in addition to the most suggestive or primary symptom; these have been designated as associated symptoms.

On the basis of the presence or absence of diarrhea as the primary symptom, the patients of this study have been separated into a diarrhea and a non-diarrhea group. The incidence of both primary and associated symptoms in each group is indicated in Table 1.

The amebae were found by various methods. In most cases, and particularly those with diarrhea, ordinary stools were examined. When these were negative, the patient was given 15 gm. of magnesium sulfate in one-half glass of water in the morning. The first stool following this purgative was discarded and the next 3 stools were examined for amebae. This was usually done at least 3 times in a suspected case and preferably not on succeeding days. In some cases during proctoscopy ulcerated areas of the bowel wall were swabbed and smears of this material examined. The incidence with which positive findings were obtained by these methods is indicated in Table 2. The cases have been divided into those with and without diarrhea, and it will be noted that the amebae were not often found in the unpurged stool in the non-diarrhea cases. Of the 10 cases

TABLE 1.—PRIMARY AND ASSOCIATED SYMPTOMS IN 54 CASES OF AMEBIASIS

DIARRHEA GROUP

Primary Symptoms	*Total Cases*
Diarrhea	31
Associated Symptoms	
Abdominal Pain	21
Arthralgia	2
Weakness	6
Weight Loss	7
Nausea	6
Vomiting	6
Anorexia	3

NON-DIARRHEA GROUP

Primary Symptoms	*Total Cases*
Fever	8
Abdominal Pain	8
Jaundice	3
Fistula-in-Ano	2
Eosinophilia	1
History Diarrhea	1
Associated Symptoms	
Abdominal Pain	6
Jaundice	1
Arthralgia	1
Nausea	7
Weakness	8
Diarrhea	5
Weight Loss	4
Myalgia	7
Constipation	4

with diarrhea who had positive unpurged stools, only 3 of these were positive on the first stool examination, and in 4 patients amebae were found only after a minimum of 3 stools had been examined and found to be negative. Thirty-seven of the 54 patients of this study were found to have stools positive for amebae after purging and of these the first purged stool specimen was positive in 18 patients. There were 4 cases in which the diagnosis was made by rectal swab: 3 cases of diarrhea after 1, 3, and 4 negative unpurged stool examinations respectively; and 1 case of fistula-in-ano after 3 negative unpurged stool examinations. Cysts were more commonly demonstrated than trophozoites in both the diarrhea and non-diarrhea group of cases. Trophozoites, when present, were usually seen in a case with diarrhea.

TABLE 2.—METHODS USED TO DEMONSTRATE ENDAMEBA HISTOLYTICA AND INCIDENCE OF CYST AND TROPHOZOITE FORMS

Type or Examination	*Patients with Diarrhea*	*Patients without Diarrhea*
Routine Stool		
Examined	19	13
Positive	10	3
Post-Cathartic Stool		
Positive on First Specimen	8	10
Positive on Later Specimens	10	9
Type of Organism Found		
Cysts Only	17	16
Trophozoites Only	5	2
Both Cysts and Trophozoites	9	5

A complement fixation test was done on all cases using an alcoholic extract of cultures of amebae as an antigen.

Proctosigmoidoscopy and barium enemas were performed on most cases.

Results. The incidence of positive complement fixation tests, proctoscopic findings and barium enemas is indicated in Table 3. In comparing the diarrhea and non-diarrhea groups, it will be noted that there was little difference in the number of cases showing positive complement fixation tests. Following treatment the complement fixation test became negative in 5 of the 16 diarrhea cases having positive tests prior to treatment and in 6 of the non-diarrhea cases. Proctoscopic findings were positive in only 4 of the non-diarrhea cases. Two of these cases showed a few small ulcers in the rectum, a third case showed a few small red punctate lesions, the fourth showed a narrow scarred rectum. The latter patient had a fistula-in-ano and had had an ileostomy for suspected ulcerative colitis. In the diarrhea group, proctoscopic examination was positive in 11 of the 31 cases. There were a variety of lesions seen: erythematous papules capped with small ulcers, red punctate lesions, and ulcers varying both in number and size. Only one patient in the non-diarrhea group had a positive barium enema, and this was a patient with abdominal pain who showed a deformed cecum. Nine of the diarrhea group of cases had abnormal barium enemas, and the most common finding was irritability or deformity of the cecum. One patient showed a tumor-like mass (proved later to be an ameboma) in the transverse colon.

Eleven patients had been treated for amebiasis on one or more occasions prior to being seen at this hospital.

Treatment of the cases in this hospital consisted of Diodoquin 1 gm. three times daily for 10 days followed by carbarsone .25 gm. three times daily for 10 days and then again, Diodoquin 1 gm. three times daily for 10 days. In addition, the earlier patients received emetine hydrochloride .06 gm. daily for 8 to 10 days, and the later cases chloroquin 1 gm. for 2 days, then 0.5 gm. for 18 days. Those cases that had recurrences were given aureomycin, bacitracin, penicillin or retention enemas of carbarsone or chiniofon in addition to a repetition of the above.

After treatment, patients were checked at 3, 6, 12, 18, 24, and 36 months. At each follow-up visit postcathartic stools were examined, and complement fixation tests were done in addition to appropriate history and physical examination.

The results of treatment in the diarrhea group of cases in terms of the length of follow-up has been indicated in Table 4. The follow-up studies that were used in this paper were conducted between December 1, 1950 and July 1, 1951. It will be noted that the duration of the follow-up periods varies from 3 months to 36 months. Of necessity the new and recently treated cases represent the patients with shorter follow-up periods. Although practically all patients reported at least partial relief of symptoms after treatment, it will be noted that the proportion of patients remaining cleared of symptoms decreased as the time interval following treatment increased. It can be seen that approximately 50% of the diarrhea cases had at least a partial persistence or re-

TABLE 3.—SUMMARY OF COMPLEMENT FIXATION TEST, BARIUM ENEMA, AND PROCTOSCOPIC FINDINGS

Laboratory or Clinical Examinations	*Total*	*Diarrhea Group*	*Non-diarrhea Group*
Complement Fixation Tests			
Total Number	54	31	23
Number Positive	32	16	16
Barium Enema Examination			
Total Number	47	31	16
Number Positive	10	9	1
Proctoscopic Examinations			
Total Number	44	28	16
Number Positive	15	11	4

TABLE 4.—STATUS OF DIARRHEA GROUP AT TIME OF FOLLOW-UP STUDY

Time after Treatment of Follow-up Study	*Total Cases*	*Cleared*	*Partially Cleared*	*Recurred*
No Follow-up	5	0	0	0
3 Months	3	2	1	0
6 Months	5	3	2	0
12 Months	5	3	1	1
18 Months	5	2	3	0
24 Months	3	1	0	2
30 Months	1	1	0	0
36 Months	4	1	2	1

currence of symptoms after treatment. Although no similar table had been made for cases with symptoms other than diarrhea, this tended to be true of them as well.

In Table 5 we have shown the ultimate fate of the primary as well as the associated symptoms at the time of the last follow-up examination. It can be seen that a recurrence of the associated symptom or symptoms occurred in almost half of the cases. A recurrence of major symptoms was also noted in approximately half of the cases.

TABLE 5.—STATUS OF SYMPTOMS AT TIME OF FOLLOW-UP STUDY

Primary Symptoms	*Total Cases*	*Cleared*	*Partially Cleared*	*Recurred or Persisted*	*Not Known*
Diarrhea	31	13	9	4	5
Fever	8	4	0	4	0
Jaundice	3	3	0	0	0
Fistula-in-Ano	2	2	0	0	0
Eosinophilia	1	0	0	1	0
Abdominal Pain	8	3	0	5	0
History of Diarrhea	1	1	0	0	0
Total	54	26	9	14	5
Associated Symptoms					
Fever	2	0	0	0	2
Jaundice	2	0	0	1	0
Abdominal Pain	27	11	4	6	6
Arthralgia	3	2	0	1	0
Muscular Aches	7	1	0	3	3
Constipation	4	0	0	0	4
Vomiting	6	5	0	1	0
Anorexia	3	3	0	0	0
Diarrhea	5	2	0	3	0
Total	59	24	4	15	15

It should be noted that a number of times a patient presented his first complaint of a certain symptom during a follow-up examination. Thus patients complained of muscle aches or anorexia and some who had not originally had diarrhea complained of it during follow-up visits several months after treatment. These complaints have not been included in Table 5.

There were 6 patients in whom amebae were found in the stool at a follow-up examination after treatment. These patients were treated a second time and amebae were again found in the stool in only one case. Those patients have been listed in Table 5 as showing a recurrence of symptoms only when there was a recurrence after the final course of treatment.

In Table 6 we have listed the additional diagnoses other than amebiasis that were made in this group of patients. In some cases the additional condition diagnosed could have accounted for the patient's major complaint. The major symptoms recurred in 12 of the 24 patients having these additional diagnoses.

Discussion. We have been concerned that we might be missing atypical cases of amebiasis. For if it is true that most of the large number of people who harbor amebae do have symptoms, amebiasis should be a common cause of disease. We have accordingly examined the stools in many patients who have symptoms that might be attributed to amebiasis other than typical dysentery, and have found amebae in

the stool in a number of them, as indicated in Table 1. In some of these cases we were convinced by the response to antiamebic therapy and by other elements in the clinical picture that amebiasis had been the probable cause of the patient's disability. Thus, we felt that one patient who had right lower quadrant pain and showed a deformed cecum by roentgenogram had had amebiasis involving the cecum; another who had recurring attacks of slight jaundice, right upper quadrant pain and fever had amebiasis involving the liver; a third with recurring fistula-in-ano who has had no further recurrence since therapy, had fistula secondary to amebiasis. But we also found a number of cases in which even though the symptoms cleared after therapy, we doubted that their cause had been amebiasis, for further study revealed a more likely cause. Further, about half of the entire group of 54 patients harboring amebae obtained only partial or temporary relief from anti-amebic therapy. Their symptoms had persisted or recurred when they were seen in follow-up examination.

Several other observers have noted that symptoms of amebiasis frequently persisted or recurred after treatment. This occurred when the complaint was diarrhea as well as when it was a less typical symptom although recurrence of the atypical complaints was more common.

Paulson and Andrews[10] treated 15 patients who had abdominal complaints other than diarrhea and who were found to have amebae in their stools. Though most patients had some initial relief from their symptoms, in the majority it did not last over 3 weeks and there was a recurrence by the end of the 6-month follow-up period in all 15. Howard[6] followed 22 patients who had abdominal complaints other than frank diarrhea. Only 6 (or 27%) of these patients considered themselves strikingly better or digestively well at the end of their 3-month or longer period of observation.

Sapero[11] followed a group of 110 hospital patients found to have amebae and indicated that at a 6 to 12 month follow-up about three-fourths had obtained some relief, but only half

TABLE 6.—DIAGNOSES MADE IN ADDITION TO AMEBIASIS THAT MIGHT EXPLAIN PATIENT'S COMPLAINTS

Major Complaint	*Diagnoses in Addition to Amebiasis*	*Total Cases*	*Status of Patient's Symptoms at Follow-up*		
			Cleared	*Not Cleared*	*Not Followed*
Diarrhea	Psychoneurosis	5	1	4	
	Peptic Ulcer	3	1	2	
	Ancylostomiasis	2	1	1	
	Chronic Alcoholism	2	1	1	
	Laennec's Cirrhosis	1	1		
	Achlorhydria	1	1		
	Diverticulosis of Descending Colon	1	1		
	Diabetes Mellitus	1	1		
Abdominal Pain	Psychoneurosis	4		3	1
	Psychoneurosis Non-visualized Gallbladder	1	1		
	Duodenal Ulcer	1	1		
Jaundice	Acute Hepatitis	2	2		
Eosinophilia	Parapsoriasis	1		1	

had been completely relieved of their symptoms.

Diarrhea has also been noticed to recur or persist after treatment. Palmer[9] states that the continuation of diarrhea following cured amebic colitis has so impressed observers in at least two army tropical disease centers that patients are routinely warned prior to treatment to anticipate this discouraging sequella. Stewart[12] also commented on the persistence of diarrhea after treatment for amebiasis. He studied 49 cases of diarrhea in men returning from the tropics in whom no etiological agent was demonstrated. He found that 45% of them had had amebic dysentery alone and 18.5% more had had amebic dysentery along with shigella dysentery.

There are several possible explanations for the recurrence of symptoms after treatment for amebiasis. One is that treatment has failed to eradicate the amebic infestation. In certain cases of chronic amebiasis, particularly those with chronic diarrhea, amebae do tend to reappear in the stools repeatedly in spite of successive courses of treatment[7]. But in the majority of cases, therapy such as used in this series can be expected to obtain excellent results[8]. Thus amebae were found in the stool at a follow-up examination after treatment in only 6 of these 54 cases. Likewise, Stewart could find ameba in only 6 of 29 patients in whom diarrhea recurred after treatment for amebiasis, even though he studied stools on 12 successive days and used sigmoidoscopic scraping and flotation techniques as adjuvants[12].

A second possibility is that symptoms that are not relieved by anti-amebic therapy are not due to amebic infestation. Many of our patients had other disorders as well as amebiasis (Table 6), and often one of these disorders could easily have caused the patient's symptoms. Thus a patient with abdominal pain also had a peptic ulcer. His pain at first was atypical in location, but later it appeared to respond to ulcer management and showed an ulcer pattern. Several patients with abdominal pain had psychoneurosis which was felt in a number to have been the cause of their symptoms. The one patient with eosinophilia had parapsoriasis, and as amebiasis is not commonly a cause of eosinophilia, it was felt that this was a more likely cause. One patient with fistula-in-ano had a scarred contracted colon and was felt to have ulcerative colitis. This might have contributed to the formation of the fistula.

Patients in the diarrhea group had the following associated diagnoses: psychoneurosis, achlorhydria, Laennec's cirrhosis, diverticulosis, alcoholism and peptic ulcer. Any of these conditions might have caused the patient's diarrhea, in which case anti-amebic therapy could not be expected to bring relief.

Even in some of the patients who did not have a recurrence of symptoms following anti-amebic therapy, it appeared that the illness responsible for their symptoms was not the amebiasis. Thus 2 patients with jaundice appeared to have infectious hepatitis and one of them was actually made worse by carbarsone. One of the patients with abdominal pain had evidence of gallbladder disease and this probably explained his pain rather than amebiasis of the liver. Craig[4] comments on and explains this type of finding: "It should always be remembered that *E. histolytica* is a common parasite of man, occurring in approximately from 8 to 10% of our population, and that because it is so common it will certainly be encountered in many patients suffering from disease conditions simulating in symptomology infection with this parasite."

In some patients who show persistence of diarrhea after treatment, no

amebae are found in the stool, and there is no other obvious diagnosis to explain the diarrhea. It has been postulated that in some of these cases an attack of amebic dysentery altered the patient's bowel function. Craig feels that there has been an actual change in the bowel wall secondary to the infection. Bercovitz[2] believes: "There are many patients who become bowel invalids because of a definite neurogenic tendency, which probably results from the original illness." We suspect that some of these patients may have had psychogenic diarrhea from the beginning and the amebae were an incidental finding.

Almost all authorities agree that in a group of patients found to have amebae in their stools there will be at least some who do not have any symptoms due to their infestation. These patients are referred to as "symptomless carriers" or as "cyst-passers," since it is the cystic form of the organism that is usually found in their stool. Although all of the 54 patients presented in this paper had symptoms that led us at first to suspect amebiasis, it would seem likely on reviewing their course that some of them also were carriers and their symptoms unrelated to their amebic infestation. In line with this was the fact that in 33 of the 54 only cysts were found in the stool.

After analysing our cases we came to feel that the finding of amebae should not be taken as a signal to stop the investigation in a patient, but rather the study for other possible causes of his symptoms should continue. He should be treated for his amebic infestation, but even if he responds to therapy, he should be followed for several years. His stools should, of course, be checked for the reappearance of the parasite, but his symptoms should also be reviewed. When symptoms recur in the absence of the parasite, it would be particularly important to examine him for diseases other than amebiasis; and he should not again be given anti-amebic therapy unless amebae are actually demonstrated in his stools.

Summary. A review of the clinical results at post-treatment follow-up examinations on 54 patients found to have amebae in their stools is presented. This group includes 31 patients who presented diarrhea as their major relevant complaint and 23 who presented a less typical complaint.

Parasites were found in the stools during follow-up visits in 6 patients.

Symptoms were at least partially relieved immediately after treatment in almost all cases, but follow-up examination revealed a recurrence of symptoms in approximately one-half of the patients.

In 25 cases it was noted that there was a condition other than amebiasis that could explain the patient's complaints.

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BACTEROIDES INFECTIONS
CLINICAL, BACTERIOLOGICAL AND THERAPEUTIC FEATURES OF FOURTEEN CASES*

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Although bacteria which are now grouped under the general title of "Bacteroides" have been encountered in various infections for many years, it is only relatively recently that they have been recognized as important pathogens for man. It is the purpose of this paper to call attention to the clinical features and the bacteriological data that have been observed during the past 6 years at the Johns Hopkins Hospital in 14 instances of infection due to these microorganisms. The apparent rarity of these infections is due in part at least to the relative difficulty of their isolation and cultivation, thus emphasizing the importance of careful bacteriological studies in atypical infections of all types.

The Bacteroides genus includes Gram-negative bacilli which do not form spores, and which are strictly anaerobic. They are normal inhabitants of the human body, being particularly abundant in the mouth, the female genital tract and the intestinal tract; in the latter they are said to outnumber even *E. coli*. There are two main species: one is the "funduliformis", so-named because of its tendency, during one phase of its growth in artificial media, to appear in ball-like forms. The other is the "fragilis", a smaller more regular bacillus, which does not occur as frequently in human infections and which is apparently less virulent. *B. funduliformis* is markedly pleomorphic, and in some phases of growth produces long filaments that may be difficult to distinguish microscopically from some of the actinomycetes. The absence of branching of these filaments, however, is an important feature in differentiating it from the latter. *Bacterium necrophorus* is mentioned as another species, but may be identical to the funduliformis. *Bacterium melaninogenicum*, so named because of its tendency to produce black pigment on hemoglobin-containing media, is another organism which has been isolated under conditions similar to the above.

These bacteria have been found in putrid, gangrenous diseases of many types and are often found in association with other anaerobes which are common inhabitants of the mucous membranes. The infections are much like those due to the anaerobic streptococcus which is one of the species frequently found in association with them.

One feature common to the Bacteroides group is the extraordinary difficulty of their isolation. It is frequently observed that smears of pus, particularly that having a putrid odor,

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contain myriads of Gram-negative bacilli as well as Gram-positive cocci. In the majority of such instances, the cocci are identified as anaerobic or microaerophilic streptococci, and are not difficult to isolate. The bacilli, on the other hand, may be "lost" after the first attempt at subculture because many strains show little, if any, growth on blood agar even when placed at once in an anaerobe jar. Such cultures, therefore, are apt to be reported as pure cultures of anaerobic streptococci unless smears have been made of the growth in Brewer's medium and pleomorphic Gram-negative bacilli noted in addition to the Gram-positive cocci.

Only some of the high points in the development of our knowledge of this bacterium will be mentioned, as there have been several good reviews[3,16,21] in recent years. Veillon and Zuber[24], as long ago as 1898, made accurate observations on a group of 25 cases of gangrenous suppuration, and found that various anaerobes were the etiologic agents. Thompson and Beaver[22] in 1932 reported 2 cases of septicemia with abscesses of the lungs, in both of which Bacteroides seemed to be responsible. Cohen[6] found that the same organism was frequently isolated from cases of lung abscess, along with other bacteria. Dixon and Deuterman[9] in 1937 encountered 6 instances of Bacteroides infection after operating for carcinoma of the colon. Symptoms of bacteremia began on the 10th to the 18th day postoperatively; 5 of the patients died and in each of these multiple liver abscesses were found. This is a significant contribution, as it shows that carcinomatous lesions in the colon may serve as foci in which this ordinarily non-pathogenic inhabitant of the intestinal tract may multiply and invade the blood stream. Dienes in 1942[8] performed some experiments with these organisms of considerable interest particularly from a bacteriologic standpoint. He described the occurrence of the "L-type" of colonies from the large bodies that are observed in cultures of Bacteroides. This finding is not specific for these bacteria, for the same author has noted the formation of L-forms in other species. Smith and Ropes[21] collected 20 cases of this infection from 1939 to 1943 at the Massachusetts General Hospital; some of these represented localized and others generalized infections. Of particular interest were 5 cases of meningitis secondary to otitis media, 4 of whom survived, and 3 of peritonitis due to ruptured appendix, all of whom survived. Lemierre[14] found the most common clinical types to be those associated with tonsillitis, otitis media, endometritis, urinary tract infections and those arising in the appendix. The latter often developed multiple liver abscesses with jaundice. In some an anaerobic streptococcus was isolated with the Bacteroides. Sulfonamides seemed of some help in several cases.

Until recently no chemotherapeutic agent had been found which was regularly effective. An example of this is shown in a large series of 47 patients reported by Beigelman and Rantz[3] in 1949; the Bacteroides was isolated in pure culture in 13 and was associated with an anaerobic streptococcus in 16. In the strains tested, they found clinical and laboratory evidence of considerable resistance to penicillin and to streptomycin. Recent reports, however, indicate that the newer antibiotics are proving to be more effective as therapeutic agents. This is borne out by the experience of McVay, Guthrie and Sprunt[15] who cured 2 cases of Bacteroides septicemia with aureomycin in 1949. These strains were sensitive to aureomycin, but resistant to penicillin, streptomycin and sulfadiazine. Another report from the same clinic[16] in 1952 corroborated this impression regarding the superiority of aureomycin, both

in vitro and clinically; the study deals with 35 cases, in 7 of which an anaerobic streptococcus was likewise found. And, bringing the subject more or less-up-to-date as far as antibiotics are concerned, Rantz[18] cured a patient with bacterial endocarditis due to Bacteroides with terramycin. This strain was found to be relatively resistant to penicillin and streptomycin, but very sensitive to terramycin. King and others[12] reported failure of aureomycin, chloramphenicol and terramycin in 2 cases.

Case Reports—Case 1.: This 18-year old colored girl (#377631) was admitted on March 2, 1946, complaining of pain in the chest, shortness of breath, and swelling of the ankles. She denied acute rheumatic fever or any more subtle manifestation of the rheumatic state. Seven or 8 months before admission the patient noted ankle edema for the first time. This progressed to the point where she stayed in bed most of the time. There had been no chills or fever to the patient's knowledge. About 3 weeks before admission the patient developed a cough productive of bloody sputum.

Admission examination revealed a temperature of 102°. She appeared to be acutely and chronically ill, was dyspneic and orthopneic. The skin and mucous membranes were studded with scores of petechiae. Several hemorrhages were seen in the left fundus oculi, none in the right. The sclerae were icteric. There was a small petechia in the center of the right tympanic membrane. There were moist rales over the lower half of both lung fields; dullness and diminished breath sounds were present at the right base. The heart was enlarged to the left with a straight left border. The first mitral and second pulmonic sounds were accentuated. At the apex there was a blowing systolic murmur transmitted into the left axilla and a presystolic rumble. The spleen tip was palpated 2 fingerbreadths below the costal margin. Pitting edema of the lower portion of the body extended to the waist. There was early clubbing of the fingers.

Laboratory data were as follows: Hemoglobin was 6.4 gm., white count 21,800 with a shift to the left in the differential. There was one plus albuminuria and gross hematuria with numerous white cells. Bilirubin was 5.6 mg. Seven blood cultures from early in the patient's hospital stay were sterile. Two blood cultures planted 4 days before death showed at 2 weeks (in thioglycollate media only) a partially anaerobic Gram-negative pleomorphic cocco-bacillus consistent with Bacteroides.

During the first week the patient had a spiking fever with peaks at 103 or 104°. On the third hospital day treatment with penicillin 1.2 million units a day was started. After 11 days of penicillin therapy, the temperature was still elevated, although not so markedly as previously. New petechiae were much less numerous. Penicillin was discontinued in an attempt to establish the bacteriologic diagnosis. By the 14th hospital day the patient had puffiness of the face, increase in leg edema, rise in the blood nonprotein-nitrogen from 35 to 57 mg., increase in hematuria. Temperature began to swing more widely. On the day before death, penicillin therapy was reinstituted but the patient developed severe pulmonary edema and died on March 23, 1946. The white count had fallen to 12,000 with penicillin therapy, but rose again to 18,300 late in the hospital course.

The pertinent necropsy findings were these: The heart weighed 400 gm. The mitral valve leaflets were scarred, rolled, and contracted with thickening and distortion of the chordae tendineae. The posterior leaflet was covered by a firm, rough, brownish vegetation which extended up onto the adjacent wall of the left atrium in an area which clearly represented a MacCallum patch. The chordae of the posterior cusp were ruptured. Histologically, large colonies of bacteria were stained in the vegetation. There was also evidence of healing in the vegetation. The spleen weighed 400 gm. and contained both recent and old infarcts. The kidneys likewise showed infarcts as well as small hemorrhages in the cortex and on the surface. The liver showed marked congestion and central necrosis but no abscesses.

Case 2. This 46-year-old white male clerical worker (#377906) was admitted on 3 occasions and died at this hospital on October 14, 1946. He was first admitted on March 4, 1946, because of fever, extreme weakness and weight loss of one year's duration. There was no clear history of rheumatic fever or its equivalents. His teeth had always been in poor condition. In January, 1945, two abscessed teeth had been extracted, and soon thereafter the patient's wife noticed that he had fever particularly at night and was generally "run-down". He was admitted to another Baltimore hospital where he was found to have fever and rheumatic heart disease with involvement of the mitral and

aortic valves. No explanation for the fever was found. The white count was normal. He was treated with sulfadiazine for about 3 weeks, with subsidence of fever and general improvement.

One week after his return to work, fever reappeared. In August, 1945, he was re-admitted to that hospital; again no explanation for the fever was discovered. Auricular fibrillation had appeared. This time the white count was 11,800 and 11,200 on two occasions. In September, 1945, he experienced a sudden severe excruciating pain in the left calf muscle lasting for about 3 weeks. In January, 1946, under the direction of the family physician the patient's wife gave him a total of 3.0 million units of penicillin over a two-weeks period. The fever disappeared to return a few days later. A second course of penicillin (2.0 million units total) over a one-week period likewise effected temporary defervescence. Throughout this time the patient had been getting progressively weaker. He lost 66 pounds in one year. During the year several abscessed teeth had been extracted. In November, 1945, his white count was 20,400. Hemoglobin was 78% in March, 66% in November, 1945.

At the time of first admission to the Johns Hopkins Hospital on March 4, 1946, the patient was found to be extremely emaciated. Temperature was 101°. There was auricular fibrillation. The skin was sallow, brownish-yellow in color, typically café-au-lait in appearance. There were fading petechiae on the anterior chest and numerous ones, large and small, about the ankles. The heart was enlarged to the left. The first mitral sound was accentuated and there were both systolic and diastolic mitral murmurs. The liver was 2 fingerbreadths below the costal margin; the spleen was felt down two fingerbreadths and was non-tender. Tourniquet test was positive.

Laboratory data included the following: After 19 days, four blood cultures were found to be positive for an organism of the genus Bacteroides. The organism was sensitive to 1.6 units of penicillin per cc. White count was 23,000, hemoglobin 7.4 gm. The urine showed a trace of albumin and numerous red cells. Non-protein-nitrogen was consistently 44 to 53 mg. P.S.P. excretion was depressed. Serum bilirubin was 1.1 mg. Serum albumin was 2.63 gm., globulin 3.43 gm., cephalin flocculation 4+. There was 11% retention of bromsulfalein after 30 minutes.

The patient was treated with 1.2 million units of penicillin a day for 9 weeks. During the week before penicillin was begun, the temperature had ranged from 100 to 102°. The temperature response to penicillin was a dramatic fall to normal where, for all practical purposes, it stayed for the remainder of his hospital stay. In general, the patient was transformed from a listless, weak, mentally retarded person who could barely turn over in bed to a cheerful healthy-appearing man who enjoyed bathroom privileges and gained 20 pounds of weight before discharge on May 28, 1946.

The patient was again in the hospital from June 11 to June 21, 1946, for dental extractions with prophylactic penicillin administration. Blood cultures were sterile. Bacteroides organisms could not be recovered from the tooth sockets.

The patient's final admission dated from October 4 to October 14, 1946, the day of death. He had developed progressive congestive heart failure and azotemia, and while in the hospital had emboli to the right arm and right lower lobe of the lung. Clinically, there was thought to be no evidence of bacterial endocarditis. The hemoglobin was now 16 gm. The serum albumin-globulin ratio was normal; cephalin flocculation was negative.

The most pertinent findings at necropsy were those in the heart. The mitral valve was stenosed and calcified. On the auricular surface there were several raised areas which were likewise calcified and appeared to be remnants of bacterial vegetations. There were similar polypoid excrescences along the free border of the aortic leaflets. Histologically, these lesions appeared to be thoroughly healed. No bacteria were stained.

COMMENT. These first 2 patients had subacute bacterial endocarditis due to Bacteroides superimposed on previously unrecognized rheumatic heart disease. We could find no reports of endocarditis due to this organism previous to the time these 2 cases were detected in 1946 and reported briefly in January, 1948[23]*. At least 2 cases[7,18] of Bacteroides endo-

* Dr. T. McP. Brown isolated these strains by inoculating each of 6 tubes of thioglycollate broth with 1 cc. of blood. No growth was detected by repeated smears of the sediment until the 19th day in one and the 20th in the other. The organisms were more coccoid than bacillary at first, but their bacillary form became apparent after subculture, and they were finally identified as Bacteroides.

carditis have been reported since 1948. The duration of the illness was probably over a year in the second case and probably several months in the first. The portal of entry was probably the dental sockets in Case 2. Bacteriologic cure was effected with penicillin in Case 2, but this drug was not given adequate trial in the first case. The temporary response to a short course of sulfonamide (Case 2) is of interest[3,4,21]. The striking leukocytosis and impaired liver function are noteworthy features which might be helpful in suggesting Bacteroides as the causative organism.

CASE 3. This 28-year old colored housewife (#417847) was admitted on April 3, 1947, because of pain in the left chest and cough of 4 days' duration. Beginning in the summer of 1946 she had had a total of 4 "fainting spells." During the attacks she would first see spots and zigzag lines in front of her eyes, then would fall (never hurting herself but having involuntary micturition, and some convulsive movements) and finally awake after a few minutes.

On admission the temperature was 100.4°. The tonsils were somewhat larger than normal and the pharynx was slightly red. The nasal mucosa appeared red and edematous. Respirations were rapid and shallow with obvious splinting of the left chest. The patient coughed frequently, producing little sputum, and gripping her left lower chest as she did so. There was very impressive tenderness over the left lower chest at the level of the 5th to 8th ribs. This was most marked in the area of slight swelling under the left breast in the region of the costal margin. This was neither fluctuant nor indurated but was very tender. At the left base posteriorly there was dullness with suppressed breath and voice sounds.

Laboratory data were as follows: White count was 18,200 with shift to the left in the differential; hemoglobin 10 gm. Roentgenograms of the chest showed a rather diffuse area of infiltration in the lower portion of the left lung. Type 6 pneumococcus was cultured from the sputum. Serum bilirubin was 1.6 mg., serum albumin 3.4 gm., globulin 4.2 gm., thymol turbidity 4.9 units, cephalin flocculation 2+, serum cholesterol 102 mg.

The day after admission signs of consolidation in the left lower lobe were definite with pectoriloquy, bronchophony, and tubular breath sounds. There were occasional rales. The patient was treated with penicillin in oil and beeswax 600,000 units a day for 4 days. In the first 24 hours the temperature rose to 102 to 103° and remained there for the first week. It was clear that there was no response to therapy. Two thoracenteses produced respectively 15 and 10 cc. of cloudy yellow fluid which contained 3500 white cells (mostly polymorphonuclears) per c.mm. but was sterile by culture. Finally, on April 10, thoracentesis posteriorly in the sixth interspace midway between the spine and posterior axillary line produced 300 cc. of very foul-smelling thick purulent material containing 240,000 to 300,000 white cells per c.mm. From this material were cultured an anaerobic streptococcus and an anaerobic actinomyces-like organism which was almost certainly Bacteroides. Five hundred thousand units of penicillin were instilled into the cavity; intramuscular penicillin 960,000 units a day was begun and continued for a total of one month. Six grams of sulfamerazine a day by mouth were started at the same time and continued for 16 days. In the first 24 hours following this successful thoracentesis and institution of antibacterial therapy there was a dramatic defervescence to normal. Subsequent films revealed a loculated collection of fluid and air in the lower posterolateral portion of the left chest. It was difficult to say whether any parenchymal lesion was present at this time. Thoracentesis was performed every other day with instillation of 0.5 million units of penicillin into the empyema cavity. The pus diminished rapidly in volume, became more fluid and lost its foul-smelling character. By roentgenogram the cavity closed slowly but steadily. Between April 18 and April 30 a bronchopleural fistula was demonstrable at the time of thoracentesis.

The white count remained very high, falling under 10,000 for the first time on April 20. The hemoglobin fell to 7.0 gm. by April 13, requiring transfusions. The patient was discharged well on May 31, 1947.

COMMENT. This was a case of lung abscess, empyema and broncho-pleural fistula in an epileptic. The infection probably resulted from aspiration from the tonsillar area. Toxic hepatitis and progressive anemia occurred. Striking leukocytosis lagged considerably behind the temperature in returning to

normal. Repeated thoracenteses, penicillin and sulfamerazine were successful.

CASE 4. This 35-year old Arabian hat designer from Peru (#427275) was first admitted on June 30, 1947, because of gangrene of the right leg. In 1936 he had had "pneumonia" and "colitis," following which he suffered from pain in the right leg aggravated by walking. It continued until admission to this hospital becoming much worse 2 weeks previously. A black area, painful to touch, developed over the right lower leg.

On admission the temperature was 102°. The patient appeared acutely ill. The pharynx was red and the tonsils enlarged and inflamed. Otherwise the principal findings were in the lower extremities. On the left no pulses were palpable below the femoral, but the foot was warm. On the right no pulses, including the femoral, could be palpated. The foot was cold and the toes cyanotic. Over the anterior and lateral surface of the lower leg just above the malleolus there was a large 10 by 15 cm. area of the skin which was black and necrotic. Surrounding this area the skin was a little edematous and tender.

White count was 5,900; hemoglobin 13.6 gm. It is interesting in the light of subsequent events that the electrocardiogram on admission revealed an S1, Q3 pattern consistent with previous pulmonary embolism. Serum cholesterol was 318 mg.

Lumbar sympathetic block on the right increased the circulation in the foot only very slightly. On July 1, 1947, right lumbar sympathectomy was performed by Dr. William E. Grose. During the procedure the right common iliac and external iliac arteries were palpated and found to be "hard as a rock" with no pulsations. The corresponding veins felt normal. On July 4, Dr. Grose performed a Callendar amputation of the right leg, since the gangrene had extended in spite of sympathectomy. Both the popliteal artery and vein were thrombosed. The pathologists interpreted the changes as those of arteriosclerosis.

Following amputation the patient's temperature which had previously remained quite constant at 102° followed a spiking course with daily swings from normal to 103°: this, in spite of a perfectly healthy appearing stump and right flank wound. On admission roentgenogram of the chest had shown changes, probably fluid, at the base of the left lung. Later films showed an increase in this. It was observed that there was increased "splenic dullness." Aspiration beneath the 11th rib produced thick pus. It was thought that the patient had a left subphrenic abscess with a sympathetic effusion above the diaphragm.

On July 15, Dr. Grose resected a portion of the 12th rib on the left. The peritoneum beneath it was glistening and the organs were sliding back and forth freely with respiration. A portion of the 11th rib was then resected and the pleural cavity was entered. About a pint of thick creamy pus was evacuated. Some of the neighboring lung appeared necrotic. Drains were left in place. The material from the empyema cavity contained Bacteroides by culture. The organism was sensitive to 31.2 units of penicillin per cc., slightly resistant to 15.6 units. It was sensitive to 250 units of streptomycin per cc., slightly resistant to 62.5 units.

Following drainage of the empyema a remarkable phenomenon occurred: the left pleural cavity became divided into two portions, the lower half making up the empyema cavity, the upper half which was isolated by adhesions filling up with clear sterile effusion following drainage of the empyema below. This effusion was massive, causing mediastinal shift with considerable respiratory embarrassment and necessitating repeated thoracenteses.

The patient's white count never exceeded 11,700. From the time the empyema was drained he was treated with 100,000 units of penicillin each three hours and 2.4 gm. streptomycin a day. By July 29th, the temperature was normal and remained normal thereafter with penicillin therapy only. The patient continued to demonstrate persistent tachycardia. On September 5th he suffered sudden onset of sharp pleuritic pain in the region of the right posterior axillary line. There was tenderness over the femoral vein in the amputation stump as well as slight tenderness over the femoral just above the knee. Under spinal anesthesia bilateral superficial femoral vein ligations were performed by Dr. Grose. Post-operatively the patient coughed up much blood and roentgenograms revealed that a parenchymal density had appeared at the base of the right lung. With the pulmonary embolus the temperature again rose and pursued a swinging course for the next 10 days returning to normal thereafter.

On September 25th, the temperature again rose, and considerable swelling of both thighs was discovered. The following day the temperature rose higher and the patient was now having pain in both inguinal regions, worse on the left. There was superficial heat of each thigh and marked tenderness to palpation above Poupart's ligament. The impression was that the patient had iliac thrombosis. Heparin was given by continuous intravenous

drip followed by Dicumarol. There was no definite evidence of pulmonary embolism at this time and the temperature was again normal in two days. The patient was fitted with a prosthesis and discharged on October 11, 1947, after 104 days in the hospital.

COMMENT. It seems likely that a local Bacteroides infection in the gangrenous leg led to thrombophlebitis with subsequent passage of infected emboli to the lungs with empyema formation. Venous thrombosis at the site of local Bacteroides infections is a well-recognized phenomenon[19] and is demonstrated by later cases in this series. Sympathectomy, amputation, vein ligation, anticoagulants, rib resection with drainage of the empyema, penicillin and streptomycin were successful.

CASE 5. This 47-year old Greek steelworker (#427648) was admitted on July 4, 1947, because of chronic cough. In 1942 he had had pneumonia for which he was hospitalized but left the hospital at a time when his physician told him his lung was not yet clear. Thereafter he continued to have some cough productive of sputum, but worked steadily and felt well generally. During the year before admission the cough increased and became more productive. He was hospitalized elsewhere in February, 1947, and told he had a lung abscess which required operation. This he refused and returned to work. The sputum increased in volume, became foul-smelling, and contained occasional streaks of blood. He was aware of mild fever and left anterior and axillary chest pain. There was no dyspnea, anorexia or weight loss.

On admission the temperature was normal and he appeared well except for frequent cough. There was minimal clubbing of the fingers. In the left axilla there were a localized decreased resonance, diminished breath sounds, and a few moist rales. The ENT consultant found evidence of chronic infection of the adenoids and tonsils. A culture obtained by bronchoscopy revealed an anaerobic streptococcus.

White count and hemoglobin were normal. Roentgenogram of the chest showed opacification of the lingula with a central translucent area consistent with abscess cavity. Bronchograms failed to reveal filling of the lingular bronchi; no bronchiectasis was identified elsewhere. Penicillin, 700,000 units per day was administered for 14 days.

On July 22, 1947, lingulectomy was performed by Dr. W. F. Rienhoff, Jr. The specimen contained thick creamy pus. Many cavities were visible grossly; all appeared to communicate with the bronchial tree. It was the opinion of the surgical pathologists that the gross and histologic picture was most consistent with chronic bronchiectasis.

Cultures of the pus in the surgical specimen grew out Bacteroides, and the same organism was recovered from cultures of pus aspirated from the left pleural cavity three days after operation. The patient was given 800,000 units of penicillin for 12 days and 2.5 gm. of streptomycin a day for 4 days postoperatively. The temperature, which had been completely normal before operation, ranged from 100° to 102° for 4 days after operation, returning to normal thereafter. The patient was discharged apparently well on August 6, 1947.

CASE 6. This 20-year old colored male janitor (#470655) was admitted on August 10, 1948, complaining of a swelling in the left anterior chest wall of 10 days' duration.

Four weeks before admission the patient developed a sore throat. On the 5th day of this illness his family physician drained creamy, blood-streaked pus from both sides of his pharynx through an incision on either side. A few days after this procedure the patient developed shooting, cramp-like pains in the abdomen with nausea, vomiting, chills and fever for two days. He was given a sulfonamide and 2 injections of penicillin.

Ten days prior to admission the patient developed a warm, tender swelling over his left anterior chest at about the level of the third rib in the midclavicular line. At the same time a similar swelling occurred on the lateral aspect of the left hip making walking difficult. Thick, creamy pus was aspirated from the chest wall abscess on 2 occasions before admission, which was prompted by a severe shaking chill on the day of admission.

Physical examination revealed an acutely ill appearing young man with a temperature of 103°, pulse 132. There was no generalized lymph node enlargement. A fluctuant swelling measuring 10 by 15 cm. was present over the left anterior chest wall just below the clavicle extending over the sternum to the anterior axillary line. Over the center of the swelling the skin was reddened, shiny and thin. The left elbow was tender, with thickening of the periarticular tissues. The natural concavity of the lateral aspect of the right hip was replaced by a tender swelling. The hip was held abducted; flexion and external rotation produced great pain. Flexion of the neck was painful; yet no mass was present there. The pharynx was benign with no discernible

scar of the previous incisions. There was a pleural friction rub at the left base posteriorly and showers of fine rales were heard at the right base. Otherwise the examination was unrevealing.

The white count on admission was 25,000 per c.mm., hemoglobin was 11.5 gm. Material aspirated from the chest wall abscess was foul-smelling, thick, greenish-white pus. Pleomorphic Gram-negative rods were stained. No acid-fast bacilli were seen. Roentgenograms of the chest showed a density with suggestive cavity in the first left anterior interspace area. Films of the clavicle showed destruction at its medial articulation. Films of the hip, spine, and elbow were normal. After 48 hours *Bacteroides funduliformis* and an anaerobic streptococcus were grown in thioglycollate media only, from the abscess pus. Blood cultures were always sterile. Aspirated material from the right hip was of the same character as that from the chest abscess. Only Bacteroides was cultured from this material. This organism was sensitive to 1.57 units of penicillin per cc., resistant to 0.78 unit.

Serum bilirubin was 2.0 mg. total; total serum protein 7.1 gm. with albumin 2.9, globulin 4.2; thymol turbidity 18.1 units; cephalin flocculation 3+, prothrombin time 24 seconds (about 50% of normal); alkaline phosphatase 7.3 units.

The patient was given 1.6 millon units of penicillin a day beginning on the third hospital day. During the first week the temperature remained 102 to 103° with corresponding tachycardia. At the end of the first week the collections of pus in the region of the right hip and left sternoclavicular joints were drained by wide surgical incision. The hip joint space itself was not involved. Within 2 days after these procedures the temperature fell to normal and remained normal for the rest of the patient's hospital stay. Penicillin was continued in the same dosage for 4 weeks. Symptomatically the patient was greatly improved. Repeated cultures of draining material were negative. The white count fell to 10,350 by the 15th hospital day and was normal thereafter.

The patient was discharged on September 9, 1948, at which time chemical studies revealed bilirubin 1.1 mg., alkaline phosphatase 4.6 units, serum albumin 4.3 gm., globulin 4.0 gm., thymol turbidity 19.5 units, cephalin flocculation negative. One month after discharge serum albumin had risen to 4.7 gm. and globulin fallen to 3.1 gm. The patient had no recurrence of any disorder which could be related to the Bacteroides infection.

COMMENT. This is a typical case of post-anginal septicemia with hectic fever, pleurisy, purulent arthritis, hepatitis, and massive subcutaneous abscesses. Internal jugular vein thrombosis may have been present to account for the tenderness in the neck. The clinical picture resembles that in the cases studied by Lemierre[14] and by Pham Huu Chi[17]. (The latter described involvement of the hip joint once, the elbow 4 times, and the sternoclavicular joint twice). In our patient surgical drainage and penicillin effected a cure.

CASE 7. This 40-year old colored sea-food restaurant cook (#476321) was admitted on September 30, 1948, after a 10-day illness characterized by weakness and jaundice. Previously he had always been well except for progressive dysphagia with regurgitation of food during the preceding 18 months.

Ten days before admission there was the onset of malaise and anorexia which led the patient to believe he was catching cold. Seven days before admission he had a shaking chill lasting about 30 minutes and followed by a drenching sweat and headache. Thereafter two chills occurred each day. Four days before admission there was onset of watery diarrhea which lasted 4 days. Epistaxis occurred on the day of admission. There was mild backache and on walking, slight aching in the knees.

On admission the temperature was 105° by rectum, the pulse rate 140 per minute. The patient was severely prostrated, dehydrated and jaundiced. He was chilling at the time of initial examination. At the right lung base posteriorly there was slight impaired percussion resonance with medium crepitant rales on auscultation. The abdomen was diffusely tender. The liver was identified extending 3 fingerbreadths below the right costal margin. The edge was tender.

Laboratory data included the following: White count was 16,700 per c.mm., with normal differential (71% polymorphonuclears). The urine positive for urobilinogen in a dilution of 1:100 and for albumin (1+). The first 3 blood cultures showed in 48 hours a growth of *Bacteroides funduliformis* which was quite sensitive to penicillin (less than 0.225 unit per cc.) and aureomycin (less than 0.19 gamma) but insensitive to streptomycin (resistant to 31.2 mcg. per cc.). Serum bilirubin was 7.6 mg., with a direct value of 5.0 mg. Alkaline phosphatase was 7.9 units

cephalin flocculation 2+, thymol turbidity 10.7 units. Chest film showed pneumonitis in the right lower lobe and a dilated redundant esophagus characteristic of achalasia.

During the first 2 days in the hospital the patient pursued a remittent temperature course, with 2 chills each day followed by temperature peak of 105°. Thereafter the temperature became unremitting at a level of 102°. Penicillin therapy (at first 1.6 million units a day) was instituted on the second hospital day. The dosage was increased progressively over the course of the next weeks to a maximum of 20.0 million units a day.

The temperature showed only a very slow decline toward normal remaining consistently above 101° for 5 weeks. It was in the 100° range during the last 10 days in the hospital when penicillin had been discontinued. There was severe pleuritic pain on the right (without evidence of significant effusion) for several days during the first week in the hospital. The white count remained between 10,000 and 17,500, being 16,240 at the time of discharge. There was a progressive development of anemia to a minimum of 26% packed red cell volume. No blood cultures after the first three were positive. At the time of discharge total bilirubin was 1.4 mg., thymol turbidity 8.6 units, cephalin flocculation negative. There was 17% bromsulphalein retention after 30 minutes.

By the 9th hospital week the patient had long since considered himself completely well and insisted on leaving the hospital. His chest film had shown progressive clearing of the parenchymal infiltration at the right base.

At home the patient remained asymptomatic and put on weight steadily during the following 6 months during which follow-up observations were made.

COMMENT. This man has achalasia of the esophagus and it is believed that his infection began with pulmonary aspiration of material from the dilated esophagus. The frequency of pulmonary complications of achalasia requires no special comment[1,13]. Thereafter, septicemia occurred secondary to aspiration pneumonia of the right lower lobe. Multiple liver abscesses probably developed. The occurrence of two daily temperature spikes is worthy of special note; the temperature chart was reminiscent of those occasionally encountered in gonococcal septicemia[10] and rare conditions such as kala-azar. The frequency of involvement of the sternoclavicular joint (see Case 6) by Bacteroides is another point of similarity to gonococcal infection. Severe hepatitis was present in this patient. There was progressive anemia and lag in the subsidence of leukocytosis which was still present at the time of discharge.

CASE 8. This 48-year old white male miner (#489281) was admitted for the first time on February 6, 1949, with draining sinuses, low grade fever, and dyspnea of 15 months' standing. He had worked underground in Kentucky coal mines from the age of 11 years. For many years he had wheezing respirations and a habitual cough, productive of about a cupful of sputum per day.

In November, 1947, the patient had pneumonia in the left lung manifested by sudden onset of chills and fever and copius bloody sputum. He was hospitalized for two weeks and treated with penicillin. From then until June, 1948, there were recurrent bouts of chills and fever, production of about a tumblerful of brownish sputum each day, and a dull ache in the left lower axilla which became intense and stabbing with deep respiration or cough. He was admitted to a Kentucky hospital on about 5 occasions and treated with penicillin with transient improvement. There were localized infiltrations in both lungs and a pleural effusion on the left, all of which led to a tentative diagnosis of tuberculosis, which, however, was never substantiated bacteriologically. In June, 1948, he suddenly vomited about a quart of very bloody fluid. His abdomen was found to be board-like. The white count was 18,000. There was 4+ test for occult blood in the stools. He was admitted to the hospital in a critical condition and emergency laparotomy performed. The peritoneum contained a large amount of bloody purulent material. Pockets of pus were found under the left diaphragm in the splenic area. A large amount of necrotic material, some of which may well have represented spleen, was removed. The patient was told that food particles were also found. Drains were left in place. The acute critical state subsided but the other symptoms previously described continued unabated and the abdomen continued to drain freely. The pus was at times foul-smelling. In December, 1948, hemoglobin was 64%, white count 7,600. On each of several admissions he was treated with penicillin

with temporary subsidence of fever. During the 15 months prior to admission to the Hopkins Hospital the patient lost 40 to 50 pounds of weight. Shortly before the Hopkins admission he had developed pedal edema for which diuretics were administered.

On admission, the patient appeared chronically ill with evidence of much weight loss. There was restriction of motion of the left chest. Dullness, diminished breath sounds and vocal fremitus were present in the lower third of the left chest. Yellowish-green pus was draining from two points in the left upper quadrant. The liver was palpated 3 cm. below the costal margin.

Laboratory data were as follows: White count was 9,240 with a normal differential, hemoglobin 15.5 gm. Serum albumin was 3.3 gm., globulin 5.4. Total bilirubin was 1.4 mg., thymol turbidity more than 32 units, cephalin flocculation 4+, and prothrombin time 23 seconds (approximately 50% of normal). The sputum was always negative for acid-fast bacilli. Films of the chest revealed a moderate sized pleural effusion on the left. There were prominent markings throughout both lung fields. Films of the abdomen after injection of the abdominal sinuses with lipiodol revealed communications with multiple cavities under the left diaphragm and in the left lateral gutter down to the level of the crest of the ilium. No definite communication with the stomach or intestinal tract was demonstrated. Charcoal taken by mouth did not appear in the sinus drainage. Electrocardiogram revealed marked right axis deviation and evidence of right ventricular hypertrophy and "strain". Eight hundred cubic centimeters of fluid were removed from the left pleural cavity at 2 sittings. The fluid had the characteristics of a transudate; it was sterile by culture. No pathogenic bacteria or fungi were discovered in the sputum or abdominal drainage on this admission.

On March 2, 1949, exploratory laparotomy with drainage of the left subphrenic abscess was performed by Dr. W. F. Reinhoff, Jr. The patient was discharged on April 14, 1949.

He was admitted for the second time on September 11, 1949. The draining areas in the abdomen had closed soon after going home. After one week at home he again experienced left upper quadrant pain. His physician aspirated the left subphrenic space but found no pus. After the procedure the patient coughed up blood streaked sputum for a time. Late in May the abdomen began to swell. By mid-July, the patient was so ill with abdominal distension, dyspnea, ankle edema, left chest pain and fever that he underwent an operation on the left chest at a Kentucky hospital. Three gallons of pus were said to have been removed. The site of the thoracotomy healed. In mid-August fever developed and the chest incision opened, discharging a large amount of pus. Soon thereafter he again entered the Johns Hopkins Hospital.

At the time of this second admission, the temperature was normal but the patient was wasted and had a florid face. In contrast to the rest of his body the abdomen was round and protuberant. He was orthopneic. There was a small draining sinus in the 8th left intercostal space in the posterior axillary line. The tongue was beefy-red with evidence of papillary atrophy. The left chest was contracted with scoliosis to that side. There was dullness to percussion posteriorly at both lung bases, more marked on the left. Breath sounds could not be heard at the left base. There was a friction rub at the right base. The second pulmonic heart sound was accentuated. The liver was a full handbreadth below the right costal margin. There was clubbing and slight cyanosis of the fingers and three plus ankle edema.

Laboratory data were in most respects identical to those on the previous admission. Serum albumin was now 2.1 gm. and globulin 6.7 gm. The urine showed a specific gravity of 1.016 to 1.022 with two plus albumin. There was 25% bromsulfalein retention in 30 minutes. Chest film showed extensive clouding in the lower two-thirds of the left lung field. There was minimal infiltration in the root and base on the right lung. Thoracentesis of the left chest yielded 350 cc. of thick greenish-gray, foul-smelling pus. Cultures revealed Bacteroides. The sensitivity of this organism to antibiotics is shown in Table 2.

On September 16, rib resection and open drainage of the left-sided empyema was performed by Dr. Rienhoff, and 750 cc. of thick foul pus were removed.

The patient was treated with aureomycin 3 gm. a day for the last 2 weeks of this hospitalization. Improvement was slow but steady. Material draining from the thoracotomy wound became no longer foul, cultures for Bacteroides became negative and the wound slowly healed. The patient was discharged home on October 8, 1949.

COMMENT. This 48-year old miner had chronic pulmonary infection and probably cor pulmonale. The course of events is not entirely clear, but it seems possible that perforation of the

gastrointestinal tract led to abdominal abscesses, with chronically draining sinuses due perhaps (not proved) to Bacteroides infection. A left pleural effusion was at first sterile and probably sympathetic in origin but was later replaced by Bacteroides empyema.

CASE 9. This 37-year old colored housewife (#165842) entered on the Surgical Service on March 17, 1949, and was transferred to the Medical Service on April 4, 1949. Her presenting complaint was a swelling in the neck. Ten days before admission there was the onset of earache on the right. With symptomatic treatment the pain disappeared. Three days before admission a swelling suddenly appeared in the soft tissues of the right side of the neck. This swelling became painful, making movement of the neck almost impossible.

On admission to the Surgical Service the temperature was 103°, pulse 140. The posterior pharnyx was beefy-red, but the tonsils were atrophic. There was a foul-smelling discharge from the right ear as well as granulation tissue in the canal. On the right side of the neck extending from the base of the skull downward into the right supraclavicular fossa and posteriorly to the midline and down over the right scapula nearly to the inferior angle there was a tender swelling. The margin was indurated over the sternocleidomastoid. The head was tilted in a manner to relax this muscle.

Skull films were negative. The right mastoid showed complete absence of pneumatized cells; the left, some clouding in the posterior cells. Chest films revealed a rounded density with a rather mottled appearance in the right upper lung field. White count was 27,000. Incision and drainage of the neck abscess was performed on March 18, and on March 20 another fluctuant area located more posteriorly was incised. On each occasion the material from the abscess was very foul-smelling. After 13 days, cultures of the draining material revealed a growth of *Bacteroides funduliformis*. Meanwhile the white count had remained between 21,000 and 29,000 and the patient's temperature was persistently 101 to 102°, in spite of penicillin, dihydrostreptomycin and sulfadiazine therapy.

At the time of transfer to the Medical Service the only change from the findings described above was evidence of a small pleural effusion on the left. Serial films revealed progressive development of effusion while in the hospital. Left thoracentesis on April 5 yielded 35 cc. of foul-smelling thick yellowish-green pus from which Bacteroides was grown in 24 hours. Anti-bacterial therapy had been stopped one week before transfer to the Medical Service; on April 6, penicillin 2.4 million units a day was begun. The temperature continued at about 103° and the white count remained between 16,000 and 22,000. Additional pus was repeatedly removed from the neck by needle and by widening of the surgical incisions, and from the left pleural cavity by thoracentesis. During the first 3 weeks in the hospital the hemoglobin had fallen from 11.0 to 7.7 gm.

In respect to sensitivity to antibiotics, the Bacteroides organisms isolated from this patient were of 2 strains: neither was sentitive to streptomycin, one was moderately sensitive to chloromycetin; one was sensitive to aureomycin and to penicillin (cf. Table 2). Aureomycin was given in a dosage of 4 gm. a day beginning on April 14. There was a rather dramatic fall in temperature to normal, drainage from the cervical abscess decreased markedly, and the empyema dried up. The white count remained over 15,000, however.

On May 5, radical mastoidectomy on the right was performed. During the procedure it was observed that the right lateral sinus was thrombosed.

Aureomycin was continued in the same dosage for a total of eight weeks. The temperature remained normal, the cervical incisions healed, and the empyema cleared. Although she was able to take the aureomycin without nausea she failed to gain weight and the white count remained high. She was observed for 2 weeks in the hospital without aureomycin. Although the clinical improvement was maintained, at discharge more than 3 months after admission the white count was still 17,800 per c.mm. The patient was never jaundiced clinically or chemically. The white count was 8,500 per c.mm. 3 weeks after discharge. The patient remained completely well during the year of close out-patient observation.

COMMENT. This case bears many similarities to Case 6. There were otitis media, mastoiditis, lateral sinus thrombosis, and probably metastatic lung abscesses, and empyema due to *Bacteroides funduliformis*. Chronic otitis media from Bacteroides has been reported several times[16,21]. The tendency

for venous thrombosis and the persistence of leukocytosis are well illustrated. Treatment with aureomycin, surgical drainage of the neck abscess, and radical mastoidectomy was successful.

CASE 10. This 52-year old colored male painter (#546491) was admitted in July, 1950, in a semistuporous condition with a story of increasing headache, drowsiness and weakness of the right arm and leg.

Two weeks before admission there was the onset of frontal headaches of a type he had never had before, together with slight chilliness, feverishness, and sweating. The headache continued with increasing severity. Two days before admission he was very drowsy, had some difficulty talking and keeping his train of thought, and remained in bed. Weakness of the right arm and leg and incontinence of urine were noted on the day before admission. On the day of admission he became unresponsive.

Admission examination revealed a comatose, thin, colored man with Cheyne-Stokes respiration. He responded to painful stimuli with slight movements of the left arm and leg. The pupils were equal and showed dilatation during periods of apnea. Hoffman and Babinski signs were demonstrated on the right side. The patient was afebrile and the blood pressure was 160/90.

The white count was 16,300 with predominance of polymorphonuclear cells. The spinal fluid was crystal clear with a pressure of over 600 mm., 7 mononuclear cells per c.mm., and one plus Pandy reaction.

The patient was thought to have either subdural hematoma or intracranial neoplasm. Burr holes were placed over each posterior frontal area. No subdural hematoma was found but on attempting to cannulate the left lateral ventricle a large abscess containing about 50 cc. of thick foul-smelling, greenish-yellow pus was encountered at a depth of about 5 cm.; 30,000 units of penicillin (in 10 cc. of solution) were instilled. While still on the operating table, he began to respond to verbal stimuli.

After returning to the ward he was placed on 18.0 million units of penicillin a day. There was a steady improvement. The material from the abscess cavity was found to contain an anaerobic gamma streptococcus and a Bacteroides organism. The streptococcus was said to be "sensitive" to penicillin, streptomycin, aureomycin, terramycin, and chloromycetin. The Bacteroides was "moderately sensitive" to streptomycin and "sensitive" to the others. At no time did the patient demonstrate much fever.

Pencillin was given for a total of 6 weeks and aureomycin 3 gm. a day for about 2 weeks. He was discharged without any abnormal findings on neurologic examination. He began to have Jacksonian fits in March, 1951, but these were satisfactorily controlled with phenobarbital.

COMMENT. The source of infection in this case of frontal lobe brain abscess could not be determined with certainty. Treatment by neurosurgical drainage, penicillin and aureomycin was successful.

CASE 11. This 13-year old white boy (#184385) was admitted with a 12-hour history of abdominal pain, which localized in the right lower quadrant, associated with anorexia, nausea, and vomiting. These symptoms had developed shortly after his brother jumped on his stomach. He had considerable direct and rebound tenderness over McBurney's point and tenderness high on the right on rectal examination. Temperature was 99.4° by mouth and the white blood count 16,400 per c.mm. Operation was immediately performed with the finding of obvious appendiceal inflammation which was confirmed, together with local peritonitis, by microscopy.

The temperature reached 101.4° (M) the day after operation declining thereafter. He was discharged on the 6th post-operative day at which time the temperature was 100°.

By 11 days after operation a fist-sized abscess had developed in the abdominal wall beneath the incisional scar. The temperature was 103°. The patient was again taken to the operating room and incision and drainage performed. About 50 cc. of thick yellow blood-tinged odorless pus were evacuated. This was located in the abdominal wall with no apparent communication with the abdominal cavity. *Bacteroides funduliformis* was cultured from the pus. He was treated with penicillin 900,000 units and streptomycin 1.5 gm. daily for three days. The temperature remained about 100° (M). for the remainder of his hospital stay. The wound slowly healed completely over the course of the next 2 months.

CASE 12. This 39-year old colored woman (#603060) was admitted on April 16, 1952, because of pain in the right side of the chest for a month. She had sustained an injury to the head in an automobile accident 7 years previously and thereafter had had grand mal epileptic attacks each one or 2 months. In the previous year she had lost about 30 lb. of weight. One year previous to admission there was onset of cough produc-

tive of a moderate amount of sputum and accompanied by malaise, feverishness, and night sweats which required her to stop her part-time work. Four months before admission the cough and volume of sputum increased. One month before admission pain began anteriorly across the lower chest on each side; the pain later became localized in the right lower chest where it had a distinctly pleuritic character. The sputum became more foul-tasting and more was produced when the patient lay on the left side.

Admission examination revealed a temperature of 101.4°. The patient appeared chronically and acutely ill. There was splinting of the right chest and signs of hydropneumothorax. There were fine rales at the left base.

Laboratory studies included the following: white count, 23,900; serum chloride 77.0 mEq./L; serum CO_2 c.p./36 mEq./L; serum albumin 2.9 gm., globulin 3.7 gm. When the patient was first seen as an out-patient a film of the chest revealed dense opacification of its entire right lower portion with a small air-fluid level. A few days later (at the time of admission) there was great increase in the size of the air-fluid level suggesting possible rupture of a lung abscess into the free pleural cavity. Thoracentesis on the right produced a liter of moderately viscid, light brown, very foul-smelling fluid. Studies of this and the sputum revealed no acid-fast bacilli, but did reveal Bacteroides and an anaerobic alpha streptococcus on culture.

The patient was placed on aureomycin 4.0 gm. a day and rib-resection with insertion of a large "garden-hose" drain into the empyema cavity was performed. The temperature fell to normal in 24 hours, yet the white count remained elevated for over three weeks. Aureomycin was continued for eight weeks, the dosage being reduced to 2.0 gm. a day during the last 2½ weeks. During that time the patient gradually gained about 8 lb. of weight and in general felt much better. A small amount of drainage was still present at the time of discharge on June 18, 1952. No communication of the remaining pleural cavity with the bronchial tree could be demonstrated.

COMMENT. The similarities of this case to Case 3 are worthy of note. Again the persistence of leukocytosis was observed.

CASE 13. This 30-year old white woman of Italian extraction (#604434) entered the Johns Hopkins Hospital on April 25, 1952, because of recurrent pain in the abdomen and right shoulder. During her teens she had suffered from epigastric pain and at the age of 18 years had been operated on in Genoa, Italy, with removal of the gallbladder and implantation of the common bile duct into the stomach. For years thereafter she was in good health. She came to the United States in 1948 and thereafter noted frequent right upper quadrant distress after eating. She had not had this pain when eating the simpler foods in Italy during and immediately following the war. In November, 1951, she developed a continuous right upper quadrant pain, which occasionally radiated through to her back, together with anorexia and fever. She vomited several times. Operation was performed at another hospital in December, 1951, but no definitive surgical procedure was done; the upper abdominal cavity showed very numerous adhesions binding down most structures in that area. The patient developed post-operative pneumonia in the right lower chest but finally left the hospital essentially asymptomatic. Late in January, 1952 fever, night sweats, and vomiting recurred. There was pain definitely related to the ingestion of fatty foods but developing 10 or 12 hours thereafter. With each period of pain jaundice developed. Five days before her admission, after a rich Italian meal, she developed severe pain in the right upper quadrant and in the right shoulder and lower chest on deep breathing. The upper abdomen became sore, and jaundice of the skin with dark urine appeared.

Examination revealed the following pertinent features: temperature 102.8°, faint icterus, high right diaphragmatic leaf with no demonstrable excursion, irregular scars of previous upper abdominal operations with fullness in the epigastrium due clearly to the liver. The liver appeared to extend about 4 fingerbreadths below the rib cage and was very tender in a localized area just to the right of the midepigastrium.

Pertinent laboratory studies included the following: white blood count 12 to 18,000; bilirubin 2.3 mg., alkaline phosphatase 17.5 Bodansky units; cholesterol 112 mg.; numerous *Giardia lamblia* organisms in duodenal aspirate.

Although no ameba forms were identified in the stool and although ascending cholangitis with liver abscesses was considered more likely than amebic abscess of the liver, a course of chloroquin was given with no effect on the temperature course. By the 11th hospital day a golf-ball size tender mass had developed on the liver to the right of the midepigastrium and during the next week it increased steadily in size until it obviously was pointing subcutaneouly. The patient was

taken to the operating room and the upper portion of a previous right abdominal incision was opened. A large abscess which was displacing the liver downward was entered and drained of "at least a liter of thick yellow pus present between the liver and diaphragm." Multiple small pockets were broken up and a large iodoform pack left in place. *Bacteroides funduliformis* was cultured from the pus.

The patient's temperature returned to normal in 12 hours and remained so thereafter. For 3 days before operation she had received penicillin 2.4 million units daily and streptomycin 1 gm. daily. These were continued for 11 days after operation. Now, some 9 months after operation and 8 since discharge from the hospital, the site of incision continues to drain small amounts of thin purulent material but the patient is essentially asymptomatic. The Bacteroides can no longer be recovered from the draining material. Even before operation the alkaline phosphatase and bilirubin had returned to normal.

COMMENT. In the past, transplant of the bile duct into the stomach has been practiced, particularly in Europe, in the treatment of peptic ulcer and of biliary tract disease. Numerous cases of ascending cholangitis have been seen in individuals with this anomalous set-up[10a]. It is clear that ascending cholangitis due to Bacteroides occurred in this patient. Ascending cholangitis due to Bacteroides has been reported to occur in individuals with the bile duct opening normally into the duodenum[20]. Hematogenous liver abscesses[2] due to Bacteroides must be commoner than the type illustrated by this patient.

CASE 14. This 39-year old white man (#603367) was first admitted on May 12, 1952, to the Surgical Service for subtotal gastrectomy in treatment of chronic duodenal ulcer. During the night following operation the blood pressure dropped to 84/60 mm. Hg from its usual value of 120/80. There was no obvious reason for this; the pressure returned to normal in a few hours. In the next 48 hours, however, the temperature went to 102° and then back to normal in a manner consistent with a myocardial infarction, which in retrospect he is believed to have had during the night following operation. On the 4th post-operative day the patient was suddenly seized wih pleuritic pain under the left costal margin, dyspnea, and cough productive of bloody mucoid sputum. There was splinting of the left chest. Electrocardiogram showed changes interpreted as posterior myocardial infarction. Progressive T wave changes occurred together with a Q_2 and Q_3 but with no S_1. It was thought likely that the patient had had a pulmonary embolus from a mural thrombus on the right side of the septum, the posterior part of which was assumed to have suffered in the myocardial infarction.

The patient was given Dicumarol. Thereafter he developed a left hemothorax. The fluid repeatedly reaccumulated, and his course during the next 6 weeks was characterized by fairly persistent elevation of temperature in the range of 101 to 102° with white counts of 15,000 to 20,000 per c.mm. and a gradual change in the character of the pleural fluid to that of rather thick pus. The "dumping syndrome" further complicated the picture. Finally 2 cultures of the pleural fluid (on June 16 and on June 19) showed a Bacteroides organism which was resistant to penicillin, but "very sensitive" to aureomycin, moderately sensitive to chloramphenicol and only slightly to terramycin (by the paper disc method). Gantrisin had been started before the Bacteroides was discovered and was continued in a dosage of 8 gm. daily with no antibiotic. Streptokinase-streptodornase was injected intrapleurally on 2 occasions and a closed drainage of the left pleural cavity by means of an indwelling catheter was instituted. (There was a severe febrile reaction with skin eruption after the second streptokinase-streptodornase injection which was done nine days after the first). Gradually the empyema cleared and the patient's general condition improved. He was afebrile off Gantrisin for 2 weeks before discharge on August 4, 1952.

COMMENT. The course of events is believed to be as follows: post-operative myocardial infarction (posterior), mural thrombus on the right side of the interventricular septum, pulmonary embolus, hemothorax from the infarction and Dicumarol, Bacteroides empyema. Surgical drainage and a sulfonamide cured the empyema. In the pre-antibiotic era treatment of Bacteroides infections with sulfonamides was occasionally accompanied by success although in most of the cases

TABLE 1.—SUMMARY OF CHIEF CLINICAL FEATURES IN FOURTEEN CASES OF BACTEROIDES INFECTION

Name, No. and Age	*Date*	*Diagnosis*	*Bacteria Isolated*	*Treatment**	*Results and Remarks*
1. V. C. 377631 18	March 1946	Rheumatic heart disease Subacute bacterial endocarditis	Bacteroides	Penicillin: 1.2 × 11 days	Therapy inadequate to control infection. Died of heart failure
2. R. H. R. 377906 46	March 1946	Rheumatic heart disease Subacute bacterial endocarditis	Bacteroides	Penicillin: 1.2 × 63 D.	Cured of infection. Died of heart failure 6 months later. Healed lesions at necropsy
3. E. M. 417847 28	April 1947	Lung abscess Empyema Epilepsy	Bacteroides Anaerobic streptococcus	Penicillin: 0.96 × 30 D. & intrapleurally Sulfamerazine: 6 × 16 D. Thoracenteses	Cured after 8 weeks.
4. J. S. 427275 35	June 1947	Gangrene of leg Thrombophlebitis Pulmonary emboli Empyema	Bacteroides	Penicillin: 0.8 × 41 D.; 0.4 × 32 D. Streptomycin: 2.4 gm × 10 D. Amputation Sympathectomy	Infection cured. Discharged after 104 days.
5. M. S. 427648 47	July 1947	Bronchiectasis Lung abscess Empyema	Bacteroides	Penicillin: 0.7 × 14 D. 0.8 × 12 D. Streptomycin: 2.5 gm × 4 D. Lingulectomy	Cured. Discharged 2 weeks after operation.
6. E. J. 470655 20	Aug. 1948	Tonsillar abscesses Abscess of chest wall Pyarthroses Liver infection	Bacteroides funduliformis Anaerobic streptococcus	Penicillin: 1.6 × 28 Days Surgical incision	Cured after 4 weeks.
7. S. H. 476321 40	Sept. 1948	Bacteremia Pneumonitis Liver infection	Bacteroides funduliformis	Penicillin: 1.6 × 12 D.; 4.8 × 9 D.; 9.6 × 49 D.; 19.2 × 13 D.	Infection controlled; slow cure during 9 weeks treatment. Apparently well 6 months later.
8 T. M. 489281 48	Feb. 1949 Sept. 1949	Subphrenic abscess Empyema Lung abscess? Liver infection Cirrhosis of liver?	Bacteroides	Surgical drainage of abdomen and chest Aureomycin: 3 gm × 14 D. Penicillin: 1.8 × 9 D.	Infection cured.
9. S. M. 165842 37	March 1949	Mastoiditis Abscess of neck Lung abscesses Empyema	Bacteroides funduliformis Anaerobic streptococcus (1 culture)	Surgical drainage Thoracenteses Mastoidectomy Penicillin: 2.4 × 5 D. 4.8 × 13 D. Aureomycin: 4 gm × 27 D. 3 gm × 25 D.	Cured. Follow up period of 1 year.
10. C. W. 546491 52	Aug. 1950	Brain abscess	Bacteroides Anaerobic streptococcus	Surgical drainage Penicillin: 18 × 30 D. 1.2 × 8 D. Aureomycin: 3 × 14 D.	Infection cured. Jacksonian epilepsy.
11. J. E. 184385 12	Jan. 1952	Acute appendicitis Abdominal abscess (post. op.)	Bacteroides funduliformis	Surgical drainage Penicillin: 0.9 × 3 D. Streptomycin 1.5 × 3 D.	Cured
12. G. J. 603060 39	Apr. 1952	Lung abscess Empyema Epilepsy	Bacteroides Anaerobic streptococcus	Surgical drainage Aureomycin: 4 × 39 D. 2 × 17 D.	Cured
13. S. Y. 604434 30	Apr. 1952	Liver abscess Subphrenic abscess	Bacteroides funduliformis	Surgical drainage Penicillin: 2.4 × 14 D. Streptomycin: 1 × 14 D.	Cured
14. T. S. 603367 39	May 1952	Myocardial infarction Pulmonary infarction Empyema	Bacteroides	Thoracenteses Closed drainage Gantrisin: 8 × 37 D.	Infection cured.

* Penicillin in million units per day. Others in grams per day.

some form of surgical treatment was combined making interpretation difficult[3,4,21].

ADDITIONAL CASE:* Currently under treatment is a case of abdominal *Actinomyces bovis* infection (#621368). Bacteroides has also been recovered repeatedly from the discharge of the fistulous tracts. The pathogenic significance of the Bacteroides has been questioned, since the fistulous tracts communicate with the sigmoid colon and perhaps originally with the urinary tract. Bacteroides is sensitive to 25 units of penicillin per cc. (resistant to 12.5 units); sensitive to 0.38 gamma of aureomycin or terramycin per cc. (resistant to 0.19 gamma). The patient is being treated with very large doses of penicillin to which the actinomycete is relatively sensitive. If clinical improvement does not ensue, the Bacteroides may be considered more important in this case than has been thought to date.

TABLE 2.—ANTIBIOTIC SENSITIVITY OF TEN STRAINS OF BACTEROIDES*

Strain from Case No.	*Pen.***	*Strep.*	*Chlor.*	*Aureo.*	*Terra.*
II.	1.6				
IV.	31.2	250			
VI.	1.57				
VII.	<0.225	62.5		<0.19	
VIII.	15.6	>125	1.9	<0.006	
IX. A.)***	15.6	625	3.1	0.38	
B.)	0.056	1250	0.75	0.19	
XI.	50	50	6.3	1.6	1.6
XII. +	0.1	6.25	0.2	0.1	0.1
XIII.	6.25	50	1.6	0.8	0.2

* Bactericidal levels.
** Penicillin measured in units per cc.; others in micrograms per cc.
*** Two strains isolated in this patient.
\+ Mixed culture employed in these patients (see text).

GENERAL CLINICAL FEATURES. Tables 1 and 3 enumerate the main features of these 14 cases. They cover most of the range of types of clinical pictures produced by these micro-organisms. The authors have not had an opportunity to observe a case of infection primary in the genito-urinary system[5]. However, a case previously reported from this hospital was an early reported example of infection of the female pelvis by an organism of this general group[11].

BIOLOGICAL OBSERVATIONS ON THREE STRAINS OF BACTEROIDES. Cultural studies were carried out on 3 of the more recently isolated strains: numbers XI, XII and XIII. One of these (XII) was never obtained in pure culture, but always in association with the anaerobic streptococcus with which it was originally recovered. This remarkable example of symbiosis persisted in spite of attempts to kill the streptococcus selectively with penicillin and other antibiotics. In several such tries with penicillin it seemed on smear that all streptococci had been removed, but subcultures showed that this organism was still present, only being killed by concentrations of the antibiotics that were likewise lethal to the Bacteroides; consequently all studies reported subsequently in which strain XII was employed refer to this combination of organisms rather than to a pure culture.

Brewer's medium proved to be satisfactory for primary isolation, subcul-

* The original isolation and bacteriological studies of the organisms in the current case were done by Mrs. Isabelle G. Schaub, whose help and advice has been greatly appreciated throughout the study.

tures and antibiotic sensitivity tests by the tube dilution technique. In this medium the growth was slow, beginning in 2 or 3 days and becoming more diffuse or granular in 4 to 6 days. Strain XI began as a cottony type of growth in 3 days, then spread diffusely throughout the medium in 4 to 7 days. Strain XIII showed a little granular and ball-like growth at the bottom of the tube in 2 days, gradually spreading through the medium. The reputed growth-enhancing effect of serum was confirmed, as 10 or 20% human serum or pleural fluid led to a much more rapid and diffuse growth. In this case abundant growth usually occurred in 24 hours, and so this serum-enriched medium was found to be much more practical for subcultures and tests with the antibiotics.

TABLE 3.—SUMMARY OF PERTINENT DATA

Types of Infection	*Number of Cases*
1. Empyema	7
2. Lung Abscess*	5
3. Liver Infection	4
4. Bacteremia	3
5. Sub. Bact. Endocarditis	2
6. Abdominal Abscesses	3
7. Other Localized Suppurations	5
8. Brain Abscess	1
9. Mastoiditis	1
Number Associated with Anaerobic Streptococcus	5

Results of Therapy

Infection Cured: 12 (85%) Died: 2 (15%)

* 1 probable case included

Another interesting finding in all 3 of these strains was that they either failed to grow or showed only very slight growth when subcultured on blood agar, even if put into an anaerobic environment immediately after removal from the liquid medium. The cultures likewise had a tendency to die rather rapidly unless transferred repeatedly.

Growth of each of these strains resulted in a foul odor, just as had been noted in the original exudate. Two of them (XII and XIII) formed gas when grown in a meat medium under a vaseline seal. Each was pleomorphic, though in varying degree; strain XIII showing the formation of the characteristic long, thread-like forms to a greater extent than the others.

ANIMAL EXPERIMENTS. Cultures of all 3 strains, one of which included the anaerobic streptococcus, were inoculated intravenously and intraperitoneally into white mice. In spite of the fact that the inocula were quite heavy the mice survived, and when killed showed no lesions in any organs. The results were likewise negative when rabbits were injected intravenously and intrapleurally, though only 6 rabbits were employed in these experiments. Failure of the cultures to infect the animals under these experimental conditions may be explained by the fact that these bacteria are most apt to establish infections when their growth is initiated in an environment containing necrotic or gangrenous tissue. In these animals, therefore, the healthy peritoneum or blood-stream is evidently capable of killing large numbers of such bacteria, so that they would appear in these tests to be avirulent. Similar conclusions may hold true for this type of microorganism in humans.

MICROSCOPIC ALTERATIONS POSSIBLY INDUCED BY ANTIBIOTICS. Strains XII and XIII were tested for their sensitivity to the antibiotics, and smears made from the tubes in which the inhibition was maximal, without actual killing. Gram stains were made of these sediments, either as they were, or spun down and washed with saline, and compared with those from the control tubes, as well as from those showing lesser degrees of inhibition. Under these circumstances it was of interest to find that the tendency to the forma-

tion of long thread-like forms was usually much more apparent than in the controls or in the other tubes. This was particularly true in the case of the penicillin series, in which smears were made from the tube showing the greatest degree of inhibition-especially in strain XIII. Stains of these smears often showed that nearly all the bacteria had assumed that peculiar shape, while only relatively few such forms were found in the controls or tubes showing less inhibition of growth. This phenomenon seemed also to occur with strain XII, though to a lesser degree. The control tubes in strain XIII showed that nearly all the bacteria were the short bacilli which usually predominate in young cultures of these organisms.

Similar effects were noted with aureomycin, and terramycin and in one test with chloramphenicol, but not in one with streptomycin. These microscopic changes are illustrated in Figs. 1 and 2. These findings suggest that under conditions of maximally inhibited growth, the bacteria may assume this peculiar shape. Such morphologic changes represent variations of their growth pattern even under normal conditions, but seem to be markedly exaggerated by this type of inhibition. Possibly this represents a more resistant growth phase of the Bacteroides, but may be analagous to the changes known to occur with other bacteria when inhibited by penicillin. If similar variations occur *in vivo,* however, it might help explain the tendency of this infection, once established, to persist in the tissues of the host, as one would expect such forms to be much more resistant to phagocytosis and possibly other natural defense mechanisms of the body.

Discussion. The present study and a consideration of other reports helps us to understand some of the peculiarities of infections due to the Bacteroides group. The remarkable increase in the number of these cases observed in recent years may be due primarily to a new awareness of the circumstances under which they are most apt to occur, so that proper cultural methods are employed. Another factor no doubt playing an important role must be that, as many of the more common infections are being controlled by chemotherapy, the proportion of these more resistant ones has increased. It seems likely that we will continue to find a greater proportion of these cases; since they show such unpredictable and variable response to antibiotics, we would not expect them to respond to the usual therapeutic regimen that is so often instituted as a routine before accurate bacteriologic observations have been made.

The course this infection is apt to take is well illustrated by our patients, and is found similar in many respects to those recorded by others. This is best shown in the tables, which indicate quite well that these micro-organisms, which are ordinarily considered secondary pathogens, under suitable conditions tend to invade the tissues and thus establish active infection. The portals through which this occurs are multiple, but those most often concerned are the nasopharynx and the respiratory, genito-urinary and intestinal tracts. Following this the bacteria may invade lymphatics or blood-stream and so produce secondary foci in many areas. Anaerobic or relatively anaerobic conditions are required for their growth at both the primary and secondary foci. When walled off, a putrid type of exudate is produced. This sequence of events holds true when the Bacteroides alone is involved and also when it is present in combination with the anaerobic streptococcus, with which it shows a remarkable degree of symbiosis.

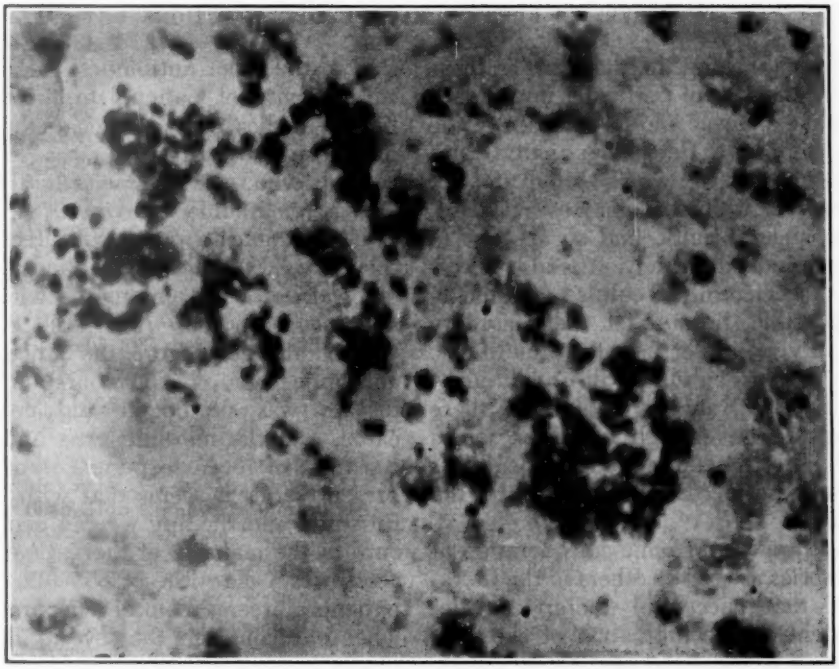

FIG. 1.—Strain XIII. Control. Demonstrates somewhat amorphous cocco-bacillary forms.

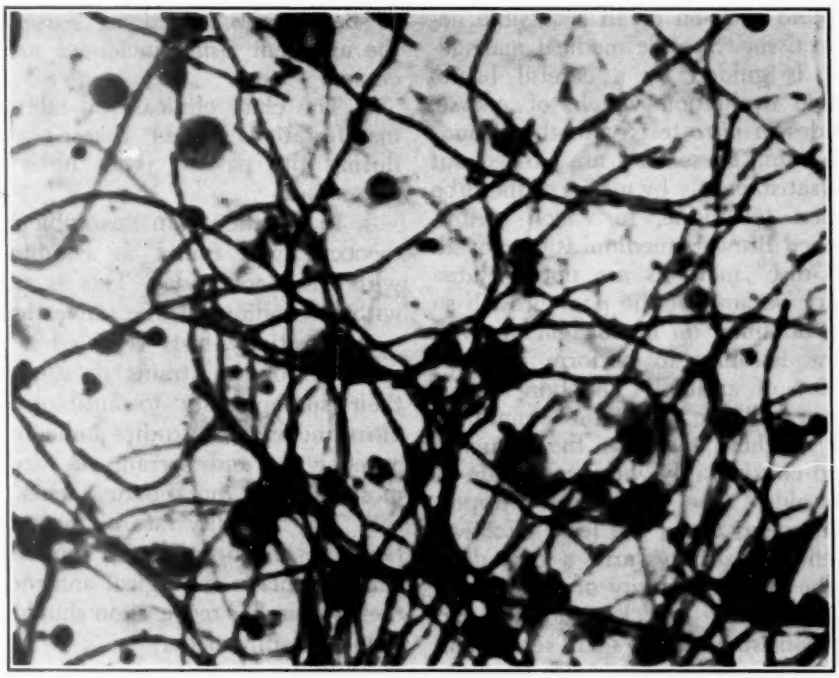

FIG. 2.—Strain XIII. Penicillin inhibition. Note predominance of long-thread forms, to many of which large round bodies are attached.

In establishing the diagnosis in these infections, it is of particular importance to make a smear and Gram stain of the pus, in order to detect the small, Gram-negative bacilli, which often do not grow out in media for several days, even anaerobically, and so are apt to be overlooked entirely. It is also necessary to perform the cultures as quickly as possible, because of their inability to survive under aerobic conditions. The primary cultures will usually grow out well in Brewer's medium, as there is apt to be enough blood or serum with the inoculum, but subcultures grow best if serum is added to the medium.

It is apparent from a consideration of this series as well as others in the literature, that successful therapy is dependent on a combination of carefully planned surgical and medical measures. The surgical technique should include adequate drainage of all walled-off foci, and excision of all accessible necrotic tissue. Specific medical management is guided by a careful bacteriologic evaluation which, of course, includes *in vitro* tests with the various antibiotics. These tests are carried out most satisfactorily by means of the tube dilution technique, in which serum-enriched Brewers medium is employed. The "disc" methods are not as satisfactory because of the poor growth so often obtained on blood agar, as well as the inability to perform accurate titration of antibiotic function in this manner. All the antibiotics should be used in these tests, as the response varied considerably with each strain of Bacteroides (see Table II). Streptomycin seems to be the least effective, penicillin sensitivity varies a great deal, but the *in vitro* activity of aureomycin and terramycin has been better and more consistent in this small series than that of the other antibiotics. The clinical response of patients to this combined medical and surgical therapy has been most gratifying, as several patients who seemed hopelessly ill have shown remarkable recoveries.

The prognosis of these infections, therefore, whether due to the Bacteroides alone or in combination with the streptococcus, has obviously been greatly altered. Before the advent of antibiotic therapy, the course of the disease was prolonged and complicated, and the mortality was high. In the light of recent experience and the present series it seems that with careful study and therapy we can expect a cure in the majority of the patients. In our series a cure rate of 85% (12 of 14 patients) seems quite encouraging, when one considers the severity of the illness in each instance.

Summary. 1. Attention is called to an increasing recognition of instances of Bacteroides infections; reasons for the apparent rising incidence are discussed.

2. The chief clinical and laboratory manifestations of 14 cases observed during the past 6 years have been presented.

3. In 5 patients an anaerobic streptococcus was found in combination with the Bacteroides. This is in line with the findings of other investigators.

4. Sensitivity tests revealed a wide variation of the strains so studied in their susceptibility to antibiotics. *In vitro* and clinical studies indicate that aureomycin and terramycin are the most effective therapeutic agents.

5. The mortality rate of these infections has been markedly reduced by carefully planned surgical and medical therapy; earlier recognition should help reduce it still further.

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ORAL UNDECYLINIC ACID IN THE PREVENTION OF THE SO-CALLED MONILIAL COMPLICATIONS SECONDARY TO THE USE OF AUREOMYCIN, CHLORAMPHENICOL AND TERRAMYCIN*

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The clinical value of the so-called wide-spectrum antibiotics in many infectious diseases has been adequately studied and reported. The side effects of nausea and diarrhea have also been noted in many reports, but the glossitis[9,22], pharyngitis[24,25], pruritus ani[12,23], pruritus vulvae[5], vaginitis[20], and possibly proctitis[3,20], and colitis[4], which attend or follow the use of these antibiotics, have been reported less often. The voluminous literature on these antibiotics does not give a clear-cut idea of the incidence of these complications, or of their persistence or severity. Some of the reports make no mention of these side effects while others suggest that the incidence may be relatively high. In the past 18 months more evidence has accumulated regarding the incidence and severity of these complications, but only an occasional publication offers means of prevention or a mode of therapy for these side effects[1,12,13].

Soon after the use of the wide-spectrum antibiotics was begun, we gained the impression that pruritus ani was a very common complication and that it was often severe and persistent. As the number of patients whom we treated with the antibiotics increased, we became aware of the additional complications of glossitis, pharyngitis, vaginitis and pruritus vulvae in what appeared to be a rather high percentage of cases and severe enough to cause us to discontinue the drug in some instances. We have seen no pulmonary[16] or cutaneous moniliasis in this series of cases.

The literature suggests several possible causes of these complications: 1) a vitamin B deficiency secondary to the elimination of the usual bowel flora[3,8,9,14,20]; 2) an overgrowth of the fungus, *Monilia (Candida) albicans*, also secondary to the elimination of the *B. coli* and other common bacteria associated with the flora of the colon[3,6,9,13,16,22,24,25]; 3) the direct stimulation of the Monilia by the antibiotic used[16,17]; 4) the combination of avitaminosis B followed by secondary in-

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fection by *Monilia albicans*[9]; 5) alteration of the host-parasite relationship[3]; 6) sensitivity to the antibiotic or the fungus[21]; 7) irritative action of the drug itself[11]. The literature contains several objections to all of these possible etiologies[10,11,22].

After having observed an occasional case which we considered clinical thrush pharyngitis and vaginitis, we theorized that the Monilia must be considered the more probable etiological agent causing many of these complications. Means of preventing these complications led us to the trial of undecylinic acid orally, since its local use as a fungicidal agent has been well established and its low toxicity when used in large doses internally has also been noted as in the treatment of psoriasis by Perlman[18] and others. On reviewing Perlman's work, we were atsonished to note that originally he used undecylinic acid orally in the treatment of the scalp fungus, *Microsporon audouini.*

Material and Methods. Undecylinic acid, which is a long-chain, unsaturated, fatty acid, occurs as an oil. It was given in capsule form in a dosage ranging from one capsule, or 0.44 gm. to 3 capsules or 1.32 gm. with each dose of the selected antibiotic, three times daily after meals. This dosage is only a small fraction of that used in psoriasis in which 15 gm. or more of undecylinic acid are often used in each 24 hours. The total dosage in our series ranged from 2.64 to 91.8 gm. In this series of cases no untoward reactions to undecylinic acid were noted. When started simultaneously with the antibiotic, we suggest one capsule of undecylinic acid (0.44 gm.) with each 250 mg. of the antibiotic.

In vitro tests with the undecylinic acid proved that the drug would inhibit the growth of Monilia but not *B.coli, Aerogenes, Proteus, Pseudomonas* or hemolytic *Staphylococcus aureus.* Oleic acid was used as a diluent in these tests and had no inhibiting or fungicidal effects on the Monilia strains in our laboratory. Similar *in vitro* tests showed that the undecylinic acid would also inhibit strains of Blastomycoses, Cryptococcus but not *Aspergillus glaucus.*

A strain of *Monilia albicans* was cultured with various dilutions of undecylinic acid and in the concentrations of 1:10 proved to be fungistatic and not fungicidal. In dilutions below this, the undecylinic acid was fungicidal. Animal experimentation on the use of oral undecylinic acid done by Newell and co-workers show that the minimum lethal dose for 50% of the guinea pigs was 8.15 ± 1.7 gm. per kilogram, thus showing the tremendous amount of the drug which might be administered to these animals orally. It is worthy of note that *in vitro* experiments suggest that sodium undecylinate is more effective than the acid in its action against the Monilia strains in our laboratory. The toxicity of this salt has not as yet been determined. Further experimentation is planned with this compound.

The patients selected for study consisted (1) of 42 adults admitted to a private hospital, including admissions to all services: medical, surgical, orthopedic, gynecological and ophthalmological; and (2) of 20 office patients in the practice of one of us. The entire staff of the hospital permitted us to use the undecylinic acid on their patients in whom the broad-spectrum antibiotics were used. The criterion used in selecting these cases was that they be given at least 1 gm. of aureomycin, chloramphenicol or terramycin daily for 5 or more consecutive days. Undecylinic acid therapy was started simultaneously with the antibiotic in the patients seen in the office. In the hospital patients, it was started several days later in order to permit the study of the changes in the fecal flora resulting from the antibiotic therapy itself and, secondly, to give a more critical trial of the protective action of the undecylinic acid. The patients were examined or questioned periodically for evidence of glossitis, pharyngitis, vaginitis, pruritus ani and proctitis during their hospital stay, and were again contacted by telephone 2 or more weeks after their discharge from the hospital so as to include any delayed reactions. The controls were the subsequent 45 cases admitted to the hospital on whom the antibiotics were again used in the dosage of 1 gm. for 5 or more days.

Laboratory studies consisted of pharyngeal and stool cultures done 3 or more days after the initiation of the antibiotic, but before starting the undecylinic acid therapy. The second culture was taken 4 or more days after the beginning of the undecylinic acid therapy. By this means, we attempted

to determine whether the undecylinic acid would cause a decrease in the number of Monilia colonies in the throat and stool cultures. These cultures were plated on blood, Endo's and Sabouraud's media; and the results were tabulated as one to four plus growth. Since only a few of these cases were under our direct care, it was very difficult to obtain complete studies on many of these patients since we could not control their discharge.

Results. We have, as yet, not seen a failure in preventing the so-called monilial complications when the undecylinic acid was started simultaneously with the antibiotic. However, this series is very small. Two patients in whom the undecylinic acid was started 3 days after the antibiotic, when contacted 2 weeks after their discharge from the hospital, reported they had had perianal itching. This was transient in one case, and occurred on the 13th day after discharge from the hospital. The patient refused therapy. The second patient had had a previous course of terramycin 6 weeks prior to his last admission, and the terramycin was again given orally in the doses of 500 mg. every 6 hours for 4 days and 250 mg. every 6 hours for an additional 3 days. Undecylinic acid was started 3 days after the antibiotic and 1.32 gm. was given for 4 successive days, at which time the patient left the hospital. The perianal itching started the day following his discharge and lasted 3 to 4 weeks and has returned intermittently ever since when he "gets nerved up". There is no previous history of pruritus ani. A culture taken 22 days after the onset of his pruritus revealed a high concentration of *B. coli,* and no *Monilia albicans* was found. This is the only case in our series in which a patient has had symptoms, and no Monilia could be recovered. We must consider these cases as 2 probable failures of the undecylinic acid therapy.

The laboratory results obtained in our studies are shown on the following tables:

TABLE 1.–ORGANISMS RECOVERED FROM STOOL 4 DAYS AFTER ANTIBIOTIC THERAPY HAD BEEN STARTED IN 32 CASES.

ORGANISM	NO. OF CASES	%
B. coli	24	75.0
Proteus	10	31.25
Pseudomonas	6	18.75
Hemolytic *Staph. aureus*	6	18.75
Monilia albicans	24	75.0

It should be noted that others have found that *B. coli* was present in significant numbers in a high percentage of stools examined[2,15], and thus was not eliminated by the oral antibiotic; nor did its presence prevent the growth of *Monilia albicans* in a high percentage of the stools studied.

TABLE 2.–COMBINATIONS OF BACTERIAL ORGANISMS RECOVERED FROM THE STOOLS 4 DAYS AFTER ANTIBIOTIC THERAPY HAD BEEN STARTED IN 32 CASES.

ORGANISMS	NO. OF. CASES	%
B. Coli and *Proteus*	6	18.75
B. coli and *Pseudomonas*	4	12.5
B. coli and Hem. *Staph. aureus*	4	12.5
Proteus and *Pseudomonas*	1	3.1

B. coli, while present in significant amounts, did not prevent the growth of the organisms which are commonly associated with the diarrheas which occur with these three antibiotics.

TABLE 3.–COMBINATIONS OF *MONILIA ALBICANS* AND BACTERIA OTHER THAN *B. COLI* 4 DAYS AFTER ANTIBIOTIC THERAPY HAD BEEN STARTED IN 32 CASES.

	NO. OF CASES	%
Monilia and *Proteus*	7	21.9
Monilia and *Pseudomonas*	4	12.5
Monilia and Hem. *Staph. aureus*	5	15.6

There appeared to be no inhibition of the growth of the Monilia by the organisms *Proteus vulgaris, Pseudomonas,* or hemolytic *Staph. aureus.*

TABLE 4.–THE RELATION OF *B. COLI* AND *MONILIA ALBICANS* IN STOOLS BEFORE UNDECYLINIC ACID.

	NO. OF CASES	%
B. coli present and Monilia present	15	50
B. coli present and Monilia absent	5	16.7
B. coli absent Monilia present	9	30
B coli absent Monilia absent	1	3.3
TOTAL	30	100

B. coli, when present in from two plus to four plus growth, did not prevent the overgrowth of *Monilia albicans.* The fungus was present in 24 out of 30 stools studied (80%).

TABLE 5.–THE RELATIONSHIP OF *B. COLI* AND MONILIA IN STOOLS AFTER ORAL UNDECYLINIC ACID.

	NO. OF CASES	%
B. coli present, Monilia present	8	36.4
B. coli present, Monilia absent	3	13.6
B. coli absent, Monilia present	10	45.5
B. coli absent, Monilia absent	1	4.5
TOTAL	22	100

The moderate decrease in the number of stools showing *B. coli* is probably due to the fact that the patients had been on the oral antibiotic 4 days longer than were those noted in Table 4, rather than that there is any suppressive action by the undecylinic acid. Monilia organisms were still present in the stools of 18 cases (82%) despite the use of undecylinic acid for 4 days. However, it should be recalled that our studies showed the fatty acid to be fungistatic rather than fungicidal.

TABLE 6.–CHANGES IN MONILIA GROWTH IN STOOLS AFTER ORAL UNDECYLINIC ACID.

	NO. OF CASES	%
Monilia growth decreased	7	41.2
Monilia growth unchanged	6	35.3
Monilia growth increased	4	23.5
TOTAL	17	100

Thus, from this table it may be seen that while the growth of Monilia was decreased in 7 cases, it was unchanged or increased in a total of 10 cases (59%).

We do not believe the number of cases shown in Tables 1 to 6 is sufficiently large to be statistically significant, but they are presented to show the fact that *B. coli* can be present in considerable numbers and there yet be a heavy growth of *Monilia albicans;* and that undecylinic acid does not clear the stool of the fungus. Similar results may be seen in the throat cultures. *Monilia albicans* was present in the throat cultures of 21 of the 37 patients who had been on the antibiotics for 4 or more days. Throat cultures taken before and after undecylinic acid were completed in 22 cases, as follows:

TABLE 7.–CHANGES IN MONILIA GROWTH IN THROAT CULTURES AFTER ORAL UNDECYLINIC ACID.

	NO. OF CASES	%
Monilia growth decreased	5	22.7
Monilia growth unchanged	3	13.6
Monilia growth increased	4	18.1
No Monilia present	10	45.4
TOTAL	22	100

Again, as in the stool cultures, it may be seen that the growth of the fungus in the throat was unchanged or increased in 7 of 12 cultures showing Monilia.

As previously mentioned, in all of these patients the undecylinic acid had been started 3 or more days following

the initial dose of the antibiotic. However, these cultures were taken after the patient had been on undecylinic acid for 4 or more days and are significant in that there has been no marked change or decrease in the Monilia growth by the added undecylinic acid.

Discussion. The mode of action of undecylinic acid in the body is unknown. We made no pharmacological studies to determine whether the undecylinic acid, which is a fatty acid, is able to pass through the intestinal tract unchanged or whether it is broken down by the intestinal enzyme system, and if so, to what degree. Similarly we found no studies by others to prove that the undecylinic acid can pass the intestinal barrier into the blood stream intact, or as a smaller but still clinically active compound. One of us took 13.2 gm. of undecylinic acid daily for 3 successive days, at the end of which the blood was drawn and plated, and it showed no inhibition of growth of laboratory strains of *Monilia albicans*. This single experiment suggests that the undecylinic acid is unable to pass the intestinal barrier unchanged in sufficient quantities to render the blood fungistatic for the *Monilia albicans in vitro*. However, if our clinical conclusions are correct, they tend to show that the oral use of undecylinic acid would prevent the glossitis, pharyngitis and vaginitis. It does seem probable that the drug does pass through the intestinal mucosa into the blood stream in some active form and thus acts systemically. Similarly, *in vivo* studies in which the oral undecylinic acid was used therapeutically in proven cases of thrush vaginitis showed a definite decrease in the growth of Monilia in the vagina. There was also a definite relief of symptoms. In addition to these observations, we have collected a small series of other cases, as yet unreported, in which the oral use of the drug appeared to be effective. This further suggests that the drug may act systemically. Perlman's work also gives evidence that this medication is able to act by way of the blood stream.

Since many of the complications secondary to these antibiotics are self-limited, ending when the antibiotics are discontinued, a true test of a therapeutic agent would be the disappearance of signs and symptoms of the complications after the initiation of therapy while the patient is still continued on the antibiotic. We are attempting to collect such a series treated with undecylinic acid to determine the possible therapeutic value of this drug in moniliasis. In our studies the undecylinic acid did not prevent the nausea or diarrhea, nor did it in any way prevent the growth of *Proteus, Pseudomonas* or hemolytic *Staphylococcus* organisms in significant quantities in the stool; and clinically it had no effect on the diarrheas which were associated with these organisms.

In regard to the etiology of the complications under discussion, our results suggest that avitaminosis B may not be a major etiological factor causing the glossitis, vaginitis, and pruritus ani, since only 2 cases of the 62 given the antibiotics also received vitamin B complex. One of our control patients, who received no undecylinic acid, developed pruritis ani and vulvae while also taking adequate quantities of vitamin B complex[22,24]. Undecylinic acid did not eliminate the growth of Monilia in the stools and pharynx following the use of aureomycin, chloramphenicol and terramycin; but if as we believe, many of these complications are due to the action of *Monilia albicans*, then it does seem that the undecylinic acid prevents the fungus from attacking the body tissues.

Caution should be used in assigning a specific cause to these complica-

tions. We have seen a few cases of pharyngitis caused by the hemolytic *Staph. aureus* as reported by Finland[7], and we have seen one patient, who had a severe pruritus ani from a previous course of aureomycin, later have a recurrence of the itching within a few hours after taking oral penicillin. We believe the latter was probably an instance of sensitivity to penicillin rather than a re-infection by the Monilia.

Summary. None of 20 patients who were given simultaneously one of the broad-spectrum antibiotics and oral undecylinic acid developed pharyngitis, glossitis, pruritus ani, pruritus vulvae, vaginitis, or proctitis.

Two of 42 patients who received undecylinic acid 3 days after the antibiotic was started developed pruritus ani. This is a failure in 3.2% of the total cases in which oral undecylinic acid was used. Twelve of the 45 control cases developed one or more of the above symptoms. This is an incidence of 26.7%.

There were no side-reactions to oral undecylinic acid, nor did it interfere with the clinical effectiveness of the antibiotic used, since a high percentage of the patients made very good recoveries from their various infections.

The undecylinic acid did not prevent the recovery of the fungus, *Monilia albicans*, from the stool or the pharynx in a high percentage of the patients who received the drug. It has not been proven that the drug acts as a fungicidal agent, but no clinical thrush occurred during its use.

If these results can be confirmed, the oral use of undecylinic acid should be considered whenever oral aureomycin, chloramphenicol or terramycin is administered. We suggest that one capsule (0.44 gm.) of the drug be given simultaneously with each 250 mg. of the selected antibiotic.

Conclusions. 1) Oral undecylinic acid apparently prevents the so-called monilial complications which occur with the broad-spectrum antibiotics in a high percentage of patients to whom it was given prophylactically. A possible mode of action is suggested.

2) Undecylinic acid does not prevent the diarrheas which occur secondary to these antibiotics, nor does it inhibit the growth of the *Proteus*, *Pseudomonas*, or hemolytic *Staphylococcus aureus* organisms.

3) Our clinical results do not support the theories that the symptoms of glossitis, pharyngitis, pruritus ani, pruritus vulvae, vaginitis or proctitis are caused by avitaminosis B, fungus sensitivity, or the direct irritative action of the antibiotic.

4) Early results suggest that undecylinic acid may be of some value in treating these complications after they have developed.

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THIRST AS A SYMPTOM*

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MOST clinicians pay little attention to thirst as a symptom unless it becomes so pronounced that it deserves the term "polydipsia". Perhaps this is largely due to the fact that we have no clear understanding of the physiological mechanisms involved, nor is there any good objective measurement of the sensation of thirst. Subjectively many times the term has become confused with a longing for a specific liquid. Perhaps as Dr. Adolph has frequently said to me, several mechanisms may be responsible for the sensation of thirst, and attempts to simplify the problem to a single mechanism will not be successful. Perhaps the man who wrote me a penny post card is right. He said: "I could save you lots of time and useless research. It seems silly to spend money to learn information so simple it cannot be comprehended".

Current thinking on the causation of thirst is confined to two general concepts: the first that the sensation of thirst is localized in the mouth, and subjectively is associated with dryness of that region, which in turn is a function of the salivary flow. Cannon strongly supported this concept and when stranded in a London hotel after World War I, deprived himself of water and demonstrated a decrease in salivary secretion in association with the thirst[2]. Adolph's observation made in the Arizona Desert showed a direct correlation between water deficit and salivary flow[1].

The second concept related to intracellular dehydration evolved from the homeostatic thinking that thirst represents an expression of the need of the body cells for fluid. Examination of various fluid patterns has shown that the one most consistently associated with thirst is that of intracellular dehydration. It might well be that the two concepts could be combined as a single concept if it were shown that a reduction in salivary flow could be produced by intracellular dehydration.

The following clinical situations have been selected for discussion and represent situations in which the symptom of thirst can be of use diagnostically.

1. Dehydration
2. Endocrine disorders such as diabetes insipidus, hyperparathyroidism, and hyperthyroidism
3. Edematous phase of cardiac failure
4. Hemorrhage and shock
5. Disturbances of salivary secretion
6. Psychiatric or emotional disturbances

Thirst is a well recognized symptom of simple dehydration. As previously mentioned, the salivary flow and dryness of the mouth appear to correlate well with the extent of dehydration[5]. However, there are certain pitfalls in assuming that when thirst is absent,

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the patient has no need for fluids. For example, sodium depletion alone, as after intraperitoneal glucose solutions[4] or sodium depletion, together with water in concentrations equal to that of the body fluids as in sweating or drainage from a Miller-Abbott tube may elicit no thirst[3,14]. Yet the reduction in plasma and extracellular volumes may be sufficient to cause a drop in blood pressure and a rapid pulse. Frequently, thirst is assuaged before the body fluids are restored to normal. For example, with dehydration on the desert, man tends to drink back only 75% of his water deficit[15]. In contrast, injection of hypertonic solutions of salt and glucose, will induce thirst even though there has been no change in the total amount of body fluids, but merely a shift of water from the intracellular to the extracellular compartment[10]. That such injections produce a drop in salivary flow is evident from Fig. 1. All these subjects were quite thirsty and drank 400 to 800 cc. of water. They couldn't understand why they were not thirsty when given .9% salt which, as can be seen from Fig. 1, produced no drop in salivary flow. In treating dehydration it should be remembered that in patients with excessive sodium loss thirst may be minimal and yet the need for fluids be great.

A good clinical example of this is the case of acute food poisoning with severe diarrhea where the patient frequently does not complain of severe thirst till the second or third day. In such patients, fluid therapy administered to the point of relieving thirst and the dryness of the mouth would be insufficient. In other instances, an increase in salt or solutes in the blood may induce thirst and produce a body need for fluids in excess of the normal requirements. For example, in case of hyponatremia, after injection of hypertonic salt the patient will request large amounts of water. This only tends to defeat the purposes of the therapy; namely, to raise the serum sodium concentration.

In diabetes insipidus the polydipsia appears to be secondary to the polyuria and represents the thirst of simple

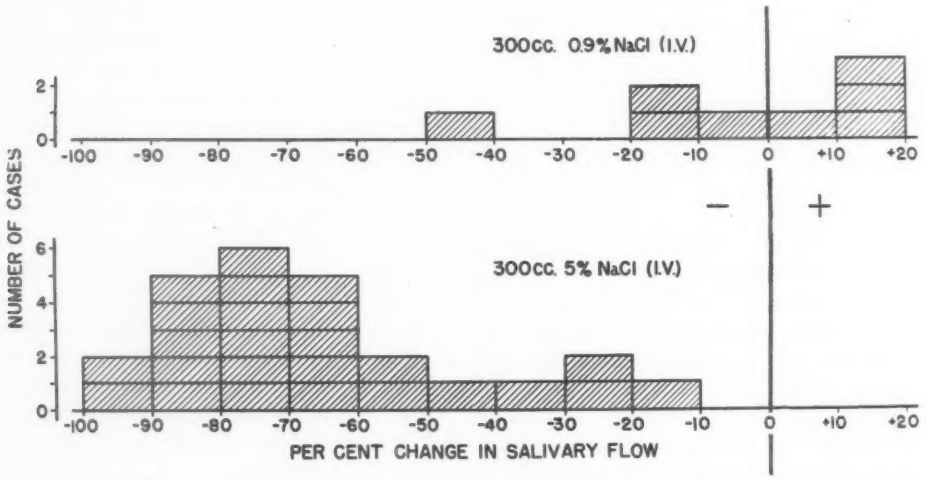

FIG. 1.—Significant decrease in salivary flow which follows the intravenous injection of 5% NaCl solutions in man.
(From Holmes and Gregersen[9])

dehydration due to excessive water loss[11]. In Table 1 are shown the fluid changes in a patient with diabetes insipidus when the urinary excretion is blocked by giving pitressin. There is an increase in weight, plasma and thiocyanate volumes, and in salivary flow: changes consistent with those observed when a patient with dehydration takes fluids. The same changes are evident when fluids are forced to the point where the intake exceeded the output (Table 1).

Kunstman, after forcing fluids (7 to 18 liters per day) for 127 days, claimed intense thirst and a continuing desire for water after the forced drinking was stopped. In dogs in which fluids (8 to 14 liters per day) were forced for 3 to 5 days, continued high intakes persisted for approximately 3 days thereafter. One wonders whether excessive water drinkers on a psychiatric basis might well drink to a point where the polyuria or polydipsia were indistinguishable from "true" diabetes insipi-

TABLE 1.—FLUID CHANGES OBSERVED IN A PATIENT WITH DIABETES INSIPIDUS (E. D.) AFTER ADMINISTRATION OF PITRESSIN (20 PRESSOR UNITS Q. 6 H.) AND AFTER FORCING OF FLUIDS WITH NO PITRESSIN (HOLMES AND GREGERSEN[11])

	Untreated	*Pitressin*	*Forced Fluids*
Fluid intake (L)	16.3	3.1	17.3
Urine (L)	15.6	2.6	15.6
Weight (kg.)	60.8	62.0	62.7
Saliva (cc.)	0.6	4.0	4.9
Plasma (cc.)	2440	3070	2800
NaScn (L)	13.8	14.8	16.8
Serum Na (mEq./L)	155	134	133
Urine NaCl (gm.) (24 hrs.)	6.3	6.5	6.4

I would like to digress in order to point out a useful diagnostic aid to differentiate the true polyuric from the excessive water drinker on a psychiatric basis. The increase in salivary flow after pitressin has been found in all 14 cases of diabetes insipidus which we have studied. (Typical examples are shown in Table 2). In contrast, in the normal person, pitressin produces a decrease in salivary flow (Table 3). Further, a dose as small as .07 pressor unit will increase the salivary flow in diabetes insipidus, although it has no effect on the salivary flow of the normal person. In 2 cases representing excessive water drinkers on a psychiatric basis, the salivary flow after pitressin was either unchanged or decreased. In the normally hydrated person, excessive water intakes of 7 to 9 liters for a 12-hour period have no effect on the salivary flow. However

dus. We have seen cases of diabetes insipidus in which cessation of the polyuria and polydipsia might well have been attributed to alleviation of psychiatric problems. The salivary test is done by blocking the nose with a nose clip to insure mouth breathing and having the patient collect all saliva in a graduated centrifuge tube during a 5-minute period. The test has also been of use in following the duration of pitressin effect in cases of diabetes insipidus where urine collections are not convenient.

Fluid measurements similar to those for diabetes insipidus have not been made in cases of hyperparathyroidism. However, preliminary studies in animals given thyroid extract would suggest a mechanism similar to that observed in diabetes insipidus; namely, a polydipsia secondary to a pronounced polyuria, and a thirst no different from that of simple dehydration[8].

TABLE 2.—INCREASE IN SALIVARY FLOW PRODUCED IN PATIENTS WITH DIABETES INSIPIDUS BY ADMINISTRATION OF PITRESSIN OR FORCING OF FLUIDS

	Salivary flow (cc./5 min.)		
Patient	*Untreated*	*Pitressin*	*Forced Fluids*
ED	0.6	4.0	4.9
TP	0.3	2.0	2.0
SD	0.2	2.6	1.0
L	0.2	2.8	1.2
BA	0.2	1.6	—

TABLE 3.—DROP OF SALIVARY FLOW IN TWO NORMAL MEN FOLLOWING THE SUBCUTANEOUS INJECTION OF 3 AND 8* PRESSOR UNITS OF PITRESSIN

	Salivary rate (cc./5 min.)			
		Minutes after Pitressin		
Subject	*Control*	*10*	*20*	*30*
B	2.0	1.5	—	0.8
B*	3.3	1.9	—	1.3
S	2.1	2.1	—	1.7
S*	2.4	—	1.7	1.3

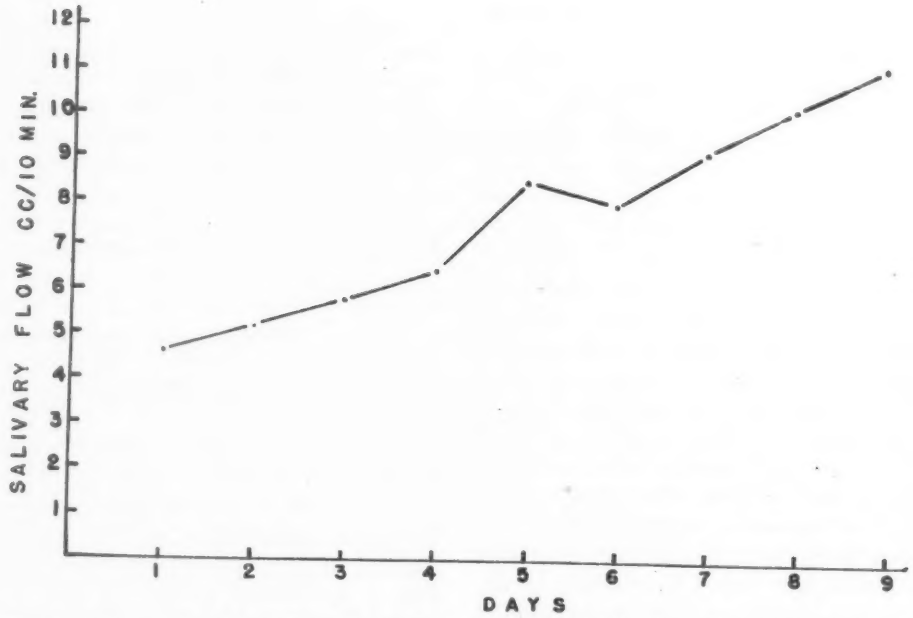

FIG. 2.—Increase in salivary flow observed in a patient with cardiac decompensation as she responded to therapy consisting of digitalis and mercurial diuretics. During this period the patient lost 12 lbs. and became edema-free.

The cardiac patient who is accumulating edema fluid frequently complains of thirst. In fact, several patients, because of their thirst have apprised us of the accumulating edema before it became evident clinically. Fig. 2 shows that the salivary flow may be reduced with cardiac decompensation and increases as the patient becomes compensated. Note that during this period the patient lost 12 pounds. It is interesting to speculate on the possible role of thirst in edema formation. The cause of thirst in cardiac edema is not clear, but may well be a result either of a reduced blood flow to the salivary glands or an intracellular dehydration.

Many of us have certainly been taught that hemorrhage invariably causes thirst. Yet to our surprise, blood bank donors showed no thirst and no drop in salivary flow, unless there was an associated circulatory collapse and a drop in blood pressure and marked vasoconstriction[12]. It will be seen from Table 4 that there was no correlation between the extent of calculated blood volume reduction and the change in salivary flow. Nor was there any correlation between the salivary response and the systolic blood pressure as long as the subject did not faint (Table 5). Thus, following simple hemorrhage (500 cc.), there was no consistent dryness of the mouth as indicated from the rate of salivary flow.

In the laboratory, dogs which have lost by hemorrhage 10 to 30% of their blood volumes show no desire for water. When epinephrine in doses insufficient to produce a drinking response in the normal animal is given at the time of hemorrhage, then there is drinking in some instances. With hemorrhage sufficient to produce shock, the dogs drink avidly[7]. Perhaps, then, with hemorrhage, thirst indicates the onset of shock and might even precede the drop in blood pressure.

Myasthenia gravis is representative of diseases in which thirst appears to be associated with a specific defect in secretion. In the untreated disease, salivary flow is reduced and returns within the normal range with prostigmine therapy. In 2 cases we have not been able to demonstrate concomitant

TABLE 4.—LACK OF CORRELATION BETWEEN CHANGE IN RATE OF SALIVARY FLOW AND THE CALCULATED DECREASE IN BLOOD VOLUME IN 50 BLOOD BANK DONORS. THE FIGURES REPRESENT THE NUMBER OF INDIVIDUALS FALLING IN EACH CATEGORY

% decrease in blood volume	*Salivary Rate*		
	Increase	*No Change*	*Decrease*
10–11	5	2	1
9–10	11	1	5
8–9	7	4	4
7–8	3	0	2
6–7	0	0	2

TABLE 5.—LACK OF CORRELATION BETWEEN CHANGES IN BLOOD PRESSURE AND IN SALIVARY RATE WHEN 50 BLOOD BANK DONORS GAVE 500 CC. OF BLOOD. THE FIGURES REPRESENT THE NUMBER OF INDIVIDUALS FALLING IN EACH CATEGORY

	Change in systolic blood pressure (mm. Hg)			
Salivary flow	*Greater than −20*	*−20 to −11*	*−10 to 0*	*0 to 20*
Increase	3	7	9	6
No Change	0	0	6	1
Decrease	3	1	2	3

changes in plasma or extracellular thiocyanate volumes.

Thirst, or at least dryness of the mouth, has long been associated with emotional disturbances, such as fear and anxiety. A college student before an important examination is a good example. This knowledge was used in the Asiatic countries as a means of picking out the guilty person through his inability to swallow a bowl of dry rice.

That the gastrointestinal tract may play an important role in thirst is shown by animal experiment. Fluids given by stomach 40 minutes prior to inducing thirst by injection of hypertonic salt solution will inhibit drinking. The same fluids given intravenously prior to salt injection do not alter the drinking response[6]. This inhibition is not abolished by superdiaphragmatic vagotomy or complete sympathectomy. A balloon distended in the stomach 40 minutes prior to salt injection will also inhibit drinking. This effect can be abolished by local application of cocaine to the stomach mucosa[13]. Curiously enough, in man, water given 40 minutes prior to injection of hypertonic salt solution prevents the decrease in salivary flow and inhibits the drinking usually observed after injection of salt[9]. This relationship between salivary flow and the stomach may be of further medical interest, since reports show an increased salivary flow in cases of peptic ulcer.

Our habit of questioning all patients about thirst has often been of value in diagnosis or in planning the course of therapy. Incidentally it always leads to a good story, frequently pointing to another illness which was not revealed by routine questioning. We have discovered that everyone has had some particular experience with thirst, which he loves to recount.

Summary. We have reviewed a number of clinical situations in which thirst may be important to the diagnosis or therapy. These are dehydration, cardiac edema, polyurias of endocrine origin, hemorrhage, diseases altering salivary secretion, and emotional disturbances. The rate of salivary secretion has been presented as one method of differentiating between true thirst and excessive water drinking. The peculiar role of the stomach in the satisfaction of thirst has been discussed. It is important to keep in mind that fluid imbalance may well represent a defect of fluid intake rather than a defect of fluid elimination.

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ELECTROPHORETIC AND CHEMICAL SERUM PROTEIN FRACTIONS IN MULTIPLE SCLEROSIS*

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The clinical signs and symptoms of multiple sclerosis are extremely protean, since any combination of lesions may be expected and almost any localization of lesions can be encountered. There are, however, certain groups of signs producing symptom-complexes of more frequent occurrence, which allow the diagnosis of multiple sclerosis in most instances. The most important signs are nystagmus, scanning speech, intention tremor, spastic paraplegia, motor and sensory disturbance, optic atrophy, emotional instability, bladder dysfunction, occasionally other cerebellar symptoms, and transient defects in muscle power. Although spastic paraplegia, speech disturbance, nystagmus and action tremor (individually or in combination) are the most characteristic presentations of this disease, any combination of defects may be manifest.

The diagnosis of multiple sclerosis, therefore, is justifiable when clinical symptoms cannot be related to a topographically focal lesion, when more than one lesion is hypothesized to account for the clinical picture, and when a history of remission and exacerbation of symptoms is demonstrable.

The clinician would be aided considerably in making a definite diagnosis if there were available some laboratory data that could differentiate multiple sclerosis from other neurologic entities of similar symptomatology but variant etiology. The purpose of this paper is to present a contribution, through the laboratory, to this differential diagnosis.

In a recent series of review articles dealing with multiple sclerosis[31,34] only those of Schumacher[31] discussed the subject of protein changes in blood or cerebrospinal fluid for this disease. He stressed the fact that such changes are not specific for multiple sclerosis, but states that the newer methods of cerebrospinal fluid protein determinations and quantitative colloidal gold testing have resulted in finding a far higher incidence of positive results than had been previously reported[21].

In a recent study of more than 2,000 patients with multiple sclerosis, Friedman and Merritt[3] observed in 78% of the cases some abnormality in the cell count, total protein and/or colloidal gold reaction of the cerebrospinal fluid. Von Storch, Harris and Lawyer[35] have used their quantitatively standardized colloidal gold reaction to show a 90 to

* This work was aided by a grant from the National Multiple Sclerosis Society, New York, N. Y.

95% occurrence of the type D curve in the spinal fluids of 100 patients with multiple sclerosis. Kabat, Glusman and Knaub[10] have developed immunochemical methods for the quantitative determination of albumin and gamma globulin in the cerebrospinal fluid of cases with multiple sclerosis. By the use of these procedures Kabat, and coworkers[9] have shown that 85% of 100 patients with multiple sclerosis showed either (a) an increased gamma globulin value or (b) an increased gamma globulin: total protein ratio in the cerebrospinal fluid. These observations were confirmed by Kabat, Moore and Landow[11] by means of electrophoretic studies of the cerebrospinal fluid of patients with multiple sclerosis. These studies showed that the electrophoretic gamma globulin is elevated in these diseases, and that this increase may occur without significant increases in the total protein or albumin. These authors did not employ the electrophoretic technique for the analysis of the serum proteins in cases of multiple sclerosis.

There has been increasing experimental evidence lately that abnormalities exist in the serum protein of patients with multiple sclerosis simultaneously with those present in the cerebrospinal fluid. For example, Schumacher's review article[31] states that Kane[12] found elevated thymol turbidity values in multiple sclerosis which were not correlated with changes in the serum globulin as determined by a salt fractionation technique. We have made, independently, similar observations in our laboratory and in addition have obtained abnormal serum protein patterns with sera from multiple sclerosis cases, which resemble those found in liver disease, employing the quantitative cephalin-cholesterol flocculation reaction of Saifer[27]. Swank, Franklin and Quastel[33] have reported abnormal plasma protein patterns in multiple sclerosis using paper chromatography.

While all these results indicate serum protein abnormalities in cases of multiple sclerosis, the procedures used are either qualitative or semi-quantitative in nature. They do serve to emphasize the need for a more *quantitative* study of the distribution of the serum protein fractions in carefully evaluated cases of multiple sclerosis, such as can be provided by the electrophoretic method. This paper presents such data for 23 cases of multiple sclerosis which appears to be the first large series of such cases studied with the electrophoretic procedure.

However, the electrophoretic technique has certain disadvantages for routine clinical work, such as requiring expensive equipment, skilled technical help and permitting only 2 or 3 determinations to be performed daily. In order to explore the possibility of using simpler techniques, we have run comparatively with the electrophoretic method, such serum protein procedures as the A:G ratio by salt fractionation, the thymol turbidity test, the quantitative cephalin-cholesterol flocculation reaction, the zinc sulfate turbidity reaction, the ammonium sulfate-sodium chloride turbidity test and the quantitative protein flocculation-ninhydrin method for gamma globulin.

In general these studies indicate marked abnormalities in certain serum protein fractions in cases of multiple sclerosis which can be detected either with the electrophoretic method or with some of the simpler protein flocculation reactions. These serum protein findings together with cerebrospinal fluid determinations and careful clinical evaluation of the cases may lead to a more accurate diagnosis for this disease, help in differentiating it from related demyelinating diseases and prove useful for the objective evaluation of any new therapeutic measures as they become available.

Methods. (a) SELECTION OF CLINICAL MATERIAL. The patients for this study were selected in accordance with the diagnostic criteria for multiple sclerosis as previously defined.

(b) ELECTROPHORETIC PROCEDURE. Samples of blood, 15 to 20 cc., were drawn before breakfast from the group of selected multiple sclerosis cases and allowed to clot at room temperature. Clear, unhemolyzed serum was obtained by centrifugation at low speeds, and if not worked on immediately, was frozen and kept in the freezing compartment of a refrigerator. It was found experimentally that such frozen sera could be kept up to a month with little or no change in their electrophoretic patterns.

Approximately 2 cc. of serum was required for each electrophoretic anaylsis. These analyses were performed by the portable Aminco-Stern electrophoresis apparatus. The optical system of the instrument is

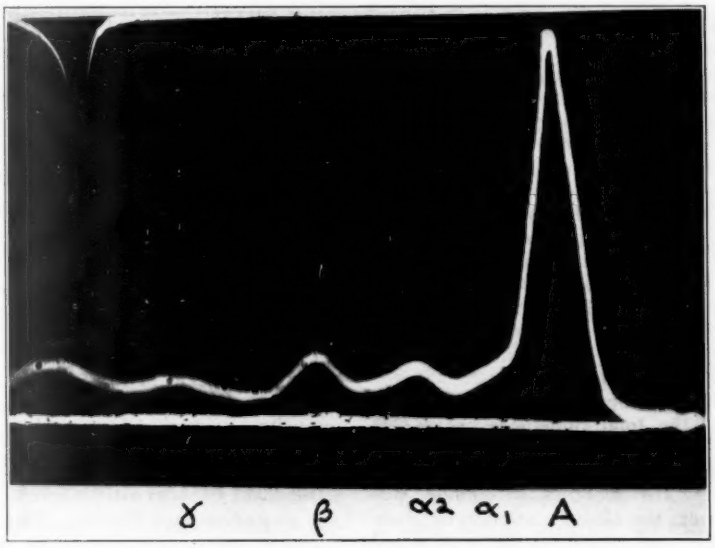

FIG. 1A.—Electrophoretic pattern for normal serum.

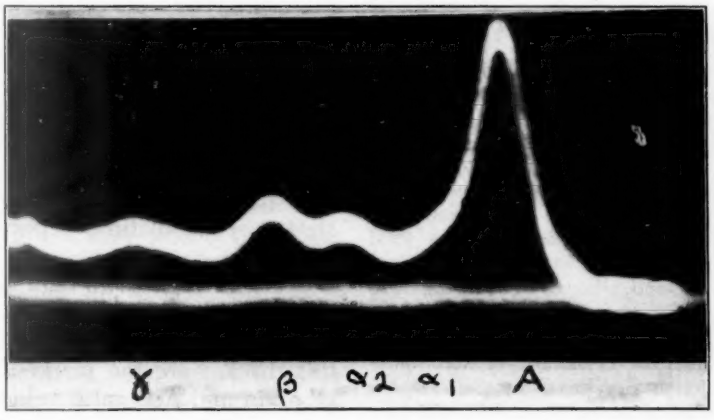

FIG. 1B.—Typical electrophoretic pattern for serum from a case of multiple sclerosis. Note the increased area under the beta and alpha-2 peaks and decreased area under the albumin peak.

based on a modification of the original Thovert method as proposed by Philpot [22] and Svensson[32]. The serum was prepared for analysis by diluting (1:3) with a barbiturate buffer (pH 8.6 to 8.7, ionic strength 0.1) and dialyzing against an excess of buffer for 48 hours in a refrigerator or in a mechanical dialyzer for at least 2 hours at 4°. A semimicro, clinical, double optical channel cell requiring a total volume of about 6 cc. was used in these experiments. Electrophoresis was carried out for about 1 hour at 14 m.a. and 2° and the patterns were photographed and traced. A typical electrophoretic pattern obtained with serum from a multiple sclerosis case is shown in Fig. 1.

All calculations were done from the ascending pattern which gave better resolution and no beta anomaly. The patterns were enlarged about 3½ times, traced on paper, and the areas measured with a planimeter. The areas representing each component were defined by the method of Longsworth[17]. The total protein values used to convert the percentage of total protein measured as areas into grams per 100 cc. of serum were obtained by the biuret procedure described below.

(c) SALT FRACTIONATION METHOD. Approximately 1 cc. of serum was used for salt fractionation of the serum proteins into albumin and globulin components and for the determination of the total protein value. A 26% solution of sodium sulfite was used for this separation as recommended by Campbell and Hanna[1], the globulin precipitate was floated off from the albumin solution by shaking with ether and centrifugation as proposed by Kingsley[14], and the albumin and total protein content determined colorimetrically with the biuret reagent of Gornall, Bardawill and David[4]. Albumin and total protein solutions, the protein content of which were determined with the micro-Kjeldahl method were used as standards. Total globulin values were calculated as the difference between the total protein and the albumin value.

(d) QUANTITATIVE PROTEIN FLOCCULATION METHODS. The photometric flocculation-ninhydrin procedures used in these experiments for the quantitative determination of serum gamma globulins and for the total serum:gamma globulin ratio values, and their chemical and clinical significance have been discussed in detail in previous publications from this laboratory[29, 30].

The quantitative cephalin-cholesterol flocculation reaction developed by Saifer[26] together with temperature variation[27] was employed to determine the serum protein patterns for cases of multiple sclerosis.

(e) QUALITATIVE PROTEIN FLOCCULATION METHODS. Tests used in these studies included the cephalin-cholesterol flocculation procedure of Hanger [5], the thymol turbidity reaction of Maclagan[18], the zinc sulfate turbidity test of Kunkel[15] and the ammonium sulfate-sodium chloride turbidity test of Huerga and Popper[7].

Results and Comments. The 23 cases of multiple sclerosis chosen for this study of their serum protein fractions were carefully selected and evaluated clinically by the Neurological Service of this hospital, using the criteria previously described. They constitute part of a larger group of cases of multiple sclerosis, and other related demyelinating diseases, now undergoing similar studies of the quantitative distribution of the various protein fractions present both in the serum and in the cerebrospinal fluid. This paper is then in the nature of a preliminary report dealing mainly with our serum protein findings and especially with the results of our electrophoretic studies.

The electrophoretic serum protein fractions in normal individuals have been extensively studied and the available data on this subject was compiled, in a paper by Reiner, Fenichel and Stern[24]. Since the instrument used in our electrophoretic studies was of a design similar to that used by these investigators, only a small series of normal sera were run by us. As our normal values for the electrophoretic protein components checked closely with those obtained by these authors[24], their larger series of 80 cases was used as a guide in determining deviations from the normal patterns and in performing the statistical calculations reported in this paper.

The electrophoretic data obtained in these studies are summarized in Tables 1, 2 and 5. The significant changes for this disease are the markedly lowered albumin and A:G ratio values, and the increased beta and alpha-2 globulin values. The alpha-1 globulin values fall

within the normal range but the gamma globulin fraction is usually slightly elevated above the normal mean value for this component. The mean values, standard deviations and the range of values obtained for these 23 selected cases of multiple sclerosis, as compared to the values obtained for normal sera are given in Table 1.

TABLE 1.—SUMMARY OF ELECTROPHORETIC DATA FOR SERA FROM MULTIPLE SCLEROSIS CASES

	Electrophoretic Data				
No. of Cases	*Albumin gm./100 cc. Mean S.D.*	*Alpha-1 gm./100 cc. Mean S.D.*	*Alpha-2 gm./100 cc. Mean S.D.*	*Beta gm./100 cc. Mean S.D.*	*Gamma gm./100 cc. Mean S.D.*
Multiple Sclerosis	3.57 ± .37	0.49 ± .11	0.80 ± .18	1.34 ± .24	1.12 ± .17
	Range	*Range*	*Range*	*Range*	*Range*
Sera (23)	2.72–4.52	0.34–0.80	0.44–1.24	0.94–1.86	0.68–1.45
Normal	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*
Sera* (80)	4.3 ± .33	0.5 ± .11	0.6 ± .17	0.9 ± .27	1.0 ± .21

* Electrophoretic data for normal sera from paper by Reiner, Fenichel and Stern.[24]

The importance of serum electrophoretic protein patterns as a possible diagnostic aid in suspected cases of multiple sclerosis is more clearly indicated by the data in Table 2. Here the 23 cases are divided into groups for each protein fraction on the basis of the number of standard deviations any particular value deviates from the mean value for that component. No value is considered abnormal unless it differs by at least *one* standard deviation from the mean value. For example, albumin has a normal mean value of 4.30 gm. per 100 cc. with a standard deviation of ± 0.33 gm. per 100 cc. Therefore, albumin values between 3.97 to 4.64 gm. per 100 cc. are considered normal, while those outside of this range are considered abnormal. Based on these criteria, 87% of the cases studied show a decrease in the albumin fraction and in the electrophoretic A:G ratio, while 74% of the cases show an increased beta globulin fraction and 65% an increased alpha-2 globulin fraction. In only 1 case out of 23 is the albumin *above* the mean normal value and in only 2 cases out of 23 are the alpha-2 globulins *below* the normal mean value. In all 23 cases the beta globulin fractions are *above* the mean value while all the A:G ratios are *below* the mean value. Even in the case of the gamma globulin fraction where only 26% of the cases show a significant increase above normal, 18 out of the 23 cases are *above* the mean normal value for this component.

To summarize the electrophoretic findings, a serum from a suspected case of multiple sclerosis which shows an electrophoretic protein pattern with a decreased albumin and A:G ratio, increased alpha-2 and beta globulin fractions, and a slightly elevated or normal gamma globulin fraction, shows a pattern typical of those obtained in about 90% of the verified clinical cases of multiple sclerosis which we have studied. The remaining 10% of the cases also show changes in *most* but not all of these electrophoretic serum protein fractions. Such a *quantitative* measure of serum protein changes in this disease compares favorably with the findings of Kabat and his coworkers[9] for cerebro-

spinal fluid, that 85% of their cases showed an abnormal gamma globulin value with their quantitative procedure. The electrophoretic serum protein findings can be used to supplement information obtained from cerebrospinal fluid protein studies better to evaluate the diagnosis or prognosis of a case of multiple sclerosis. Such studies are presently in progress at our laboratory and have already resulted in eliminating the diagnosis of multiple sclerosis in a number of questionable clinical cases.

While the electrophoretic method is invaluable for any fundamental study of quantitative serum protein fractions in the disease state, its usefulness for routine clinical work is rather limited, for reasons previously discussed. It was for this reason that it was decided to investigate, simultaneously with these electrophoretic studies, various qualitative and quantitative protein flocculation reactions as a possible simple means of detecting such serum protein changes in multiple sclerosis. With the advent of the electrophoretic technique, Maclagan[19] was able to study the chemical basis of a number of the more commonly used protein flocculation reactions. His conclusions, that these tests reflect changes primarily in the gamma globulin fraction but are also influenced by changes in the other protein fractions as well, have been confirmed by more recent investigations[8,38]. The entire field of protein flocculation reactions and its physicochemical and clinical significance has been recently reviewed by one of us[28].

TABLE 2.—DISTRIBUTION OF ELECTROPHORETIC PROTEIN FRACTIONS OF SERA FROM MULTIPLE SCLEROSIS CASES

Protein Fraction		Electrophoretic Values gm. per 100 cc. Serum					*% of Cases at least 1 S.D. removed from normal Mean Value*
Albumin		2.95–3.28	3.29–3.62	3.63–3.96	3.97–4.30*	4.31–4.64	20/23 = 87%
	No. of Cases	5	9	6	2	1	
Alpha-2 Globulins		0.43–0.60*	0.61–0.78	0.79–0.96	0.97–1.14		15/23 = 65%
	No. of Cases	2	6	12	3		
Beta Globulins		0.90*–1.17	1.18–1.45	1.46–1.73	1.74–2.01		17/23 = 74%
	No. of Cases	6	11	4	2		
Gamma Globulins		0.58–0.79	0.80–1.00*	1.01–1.22	1.23–1.44		6/23 = 26%
	No. of Cases	1	4	12	6		
A/G Ratio		0.62–0.78	0.79–0.95	0.96–1.12	1.13–1.29		20/23 = 87%
	No. of Cases	3	8	9	3		

* Mean value for normal sera. All the values in this table are grouped in terms of the standard deviation (S.D.) from this mean value as given in Tables 1 and 5 for each component.

The data obtained for the sera from the 23 cases of multiple sclerosis by some of the more commonly employed protein flocculation reactions are given in Table 3. The greatest number of positive tests for this series (66%) is given by the cephalin-cholesterol flocculation reaction of Hanger[5]. This rather high figure may appear surprising in view of the rather small increase in the

gamma globulin fraction as shown by the data in Tables 1 and 2. However, Hanger[6] has suggested that in some diseases, for example, viral hepatitis, a positive cephalin-cholesterol flocculation reaction may result from a decrease in the flocculation inhibiting properties of the serum *albumin* fraction due to its chemical modification. Such a large percentage of positive findings may then reflect changes in the electrophoretic serum albumin for cases of multiple sclerosis rather than gamma globulin changes. Maclagan and Bunn[20] have shown experimentally that alpha and beta globulin from disease sera, for example, hepatitis, also show flocculating properties with the cephalin-cholesterol reaction. Since both these fractions are elevated in almost all the multiple sclerosis cases this, too, could account for the high percentage of positive tests obtained with the Hanger method.

The thymol turbidity reaction of Maclagan[18] has been found to be positive in about 30% of the sera from cases of multiple sclerosis. This test appears to involve a mechanism fundamentally different from the cephalin-cholesterol flocculation reaction according to both clinical[36] and laboratory findings[23]. The fractions responsible for this reaction have been investigated by the electrophoretic technique by several groups of workers[20,2,16] and there is general agreement that a beta globulin, or a beta lipoprotein fraction is responsible for the positive thymol turbidity reaction. Since, as is shown in Table 2, the rather marked increase in the serum beta globulin fraction for most of the cases of multiple sclerosis would account for the 30% positive thymol turbidity results found in this disease.

The zinc sulfate turbidity test of Kunkel[15] gives about 20% positive results for sera from the 23 cases of multiple sclerosis. The zinc sulfate turbidity was proposed by its originators as a quantitative measure of serum gamma globulin content. However, Huerga and his coworkers[8] have found that the zinc sulfate turbidity values do not correlate well with either electrophoresis or the protein content of

TABLE 3.—QUALITATIVE PROTEIN FLOCCULATION DATA FOR SERA FROM MULTIPLE SCLEROSIS CASES

No. of Cases	*Thymol Turbidity* Units*	*Zinc Sulfate Turbidity* Units*	*Ammonium Sulfate Sodium Chloride Turbidity* Units*	*Cephalin-Cholesterol Flocculation 24 hour reading*
	Mean S.D.	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*
Multiple Sclerosis (23)	1.6± .9	4.4±1.5	1.9± .75	1–2+
	Range	*Range*	*Range*	*Range*
	0.5–4.4	1.1–6.8	0.7–3.3	0–4+
	% above Normal	*% above Normal*	*% above Normal*	*% above Normal*
	29%	21%	8.3%	66%
Normal Sera**	0–2	1–6	1.2–3	0 – ±

* Turbidity values in this table are all expressed in Maclagan units (Brit. Med. J., **2**, 197, 1947) and should be multiplied by two to conform to the commonly used Shank and Hoagland units (J. Biol. Chem., **162**, 133, 1946).

** Normal values listed in this table are those established by the originators of the methods used and then confirmed in our routine biochemical laboratory.

the centrifuged zinc-protein precipitates. They attribute this to the influence of albumin and lipid components present in the sera. Abnormalities in the electrophoretic albumin and beta globulin (or beta lipoprotein) fractions present in multiple sclerosis sera could then account for the 20% positive findings with the zinc sulfate turbidity reaction.

cases. The increasing specificity of the ammonium sulfate-sodium chloride turbidity method for gamma globulin would then account for the small number of positive findings (8%) obtained with this method.

While the *qualitative* protein flocculation reactions, described above, are simple to perform and may give clinically useful information, their use

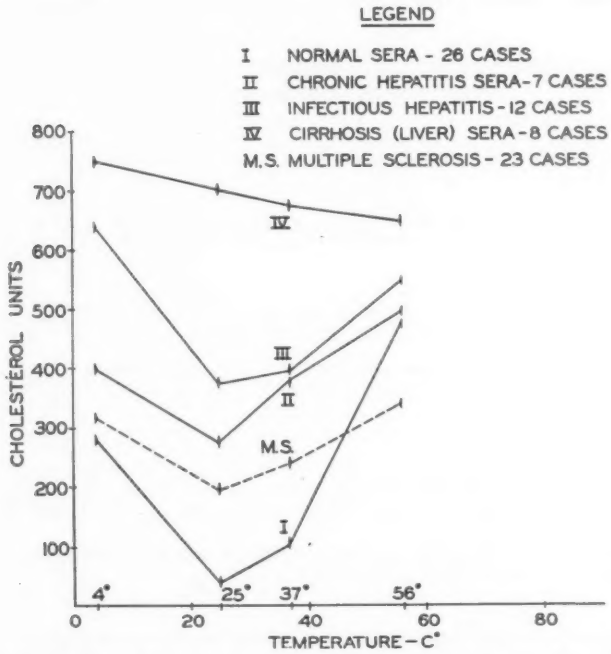

FIG. 2.—Temperature curves of average cholesterol unit values obtained for sera from various kinds of liver disease as compared with that obtained for the 23 cases of multiple sclerosis.

Huerga and his coworkers[7] have applied the salt fractionation method of Wolfson *et al.*[37] to the turbidimetric estimation of serum gamma globulin. The measured turbidities were found to be independent of albumin and lipid content of the sera, but the method only recovered 80% of added gamma globulin. As seen in Tables 1 and 2, there is only a slight increase in the gamma globulin fraction in the sera from most of the multiple sclerosis

is subject to a number of limitations. Since results are expressed qualitatively as turbidity units, or as in the case of the cephalin-cholesterol reaction from 0 to 4+, only gross changes in the clincial condition of the patient can be detected. In order to determine slight variations in the amount of flocculated material formed in the cephalin-cholesterol reaction, Saifer[26] employed a *quantitative* cholesterol method to determine the total cholesterol content

of the centrifuged flocculated complex. Results were expressed in cholesterol units and a range of values from 0 to 102 units obtained for normal sera. It was shown in the case of liver disease sera that many cases which qualitatively were read as 0 to 1+ and considered negative with the Hanger reaction gave values far in excess of 100 cholesterol units with the quantitative method. These results were confirmed in a recently published paper by Kibrick and his coworkers[13] using a turbidimetric procedure. In subsequent publications[27], this quantitative method was employed in conjunction with temperature variation to study the protein patterns obtained both with normal sera and with sera from patients with varying degrees of liver damage. Characteristic curves were obtained with each kind of disease as compared to normal sera, as shown in Fig. 2, and it was found that as the patient improved both his serum protein pattern and his cholesterol unit value tended to return to normal.

Because of the large percentage of positive results obtained with the qualitative cephalin-cholesterol flocculation reaction of Hanger, it was decided to apply the quantitative cephalin-cholesterol method with temperature variation[27] to the study of the sera from the 23 cases of multiple sclerosis. The average cholesterol unit values obtained in these runs are also shown in Fig. 2. It can be seen that the pattern obtained with this test for multiple sclerosis sera closely resembles that obtained for cases of chronic hepatitis. These average values may be somewhat misleading as 44% of the cases give a Type III pattern (similar to infectious hepatitis), 44% give a Type II pattern (similar to chronic hepatitis) and only 12% give a normal serum pattern with this test. It is interesting to note that again an abnormal serum protein pattern is obtained in about 88% of the sera from the multiple sclerosis cases with this procedure.

From the foregoing discussion, the lack of a quantitative correlation between any of the previously discussed protein flocculation reactions and any particular electrophoretic protein fraction, for example, gamma globulin, is apparent. Even in the case of the ammonium sulfate-sodium chloride turbidity method of Huerga and Popper[7], the originators recover only 80% of added gamma globulin. Ricketts and coworkers[25] have reported errors of 20% for normal sera, and as much as 100% for disease sera in a comparison between this method and the electrophoretic procedure for gamma globulin. Recently we have developed in this laboratory a quantitative protein flocculation-ninhydrin method for serum gamma globulin[29,30] which checks the electrophoretic method within ± 5% over a wide range of values. These papers also discuss the determination and clinical significance of the total serum gamma globulin values and a new clinical entity $\frac{\text{(total serum-gamma globulin)}}{\text{(globulin clot-gamma globulin)}}$ ratio. Values obtained with these quantitative protein flocculation-ninhydrin methods for the sera from the 23 cases of multiple sclerosis are summarized in Table 4, together with values obtained for normal sera. The rather exact correspondence between the globulin clot-gamma globulin value and the electrophoretic gamma globulin value is clearly shown in this table. While the finding of a normal, or slightly elevated, gamma globulin value in cases of multiple sclerosis is essentially a negative one, preliminary studies done at this hospital indicate that such a finding may be important in differentiating this disease from cases of post-encephalitic Parkinsonism where an elevated gamma globulin value is frequently found. The results in Table 4 also show that there is a significant in-

crease in the total serum-gamma globulin value, reflecting the rather marked increase in the electrophoretic beta globulins for the multiple sclerosis cases. There is a slight rise in the electrophoretic $\frac{(\text{beta} + \text{gamma})}{(\text{gamma})}$ ratio as compared to normal sera. The $\frac{(\text{total serum})}{(\text{globulin clot})}$ flocculation ratio remains normal as the rise in the total serum-gamma globulin value is offset by the rise in the globulin clot-gamma globulin value for the sera from the multiple sclerosis cases.

The results shown in Table 5 give the average values obtained for total protein and A:G ratio with the sera from the 23 cases of multiple sclerosis employing the sodium sulfite-salt fractionation-biuret method as previously described[1,14,4]. These results show that the total protein values for this disease fall within normal limits. Even with this less refined procedure, there is a significant fall in the albumin and A:G ratio, and a significant rise in the total globulins as compared with normal sera. With this less accurate procedure only one case had an A:G value above 2.0, while 8 cases out of 23 had A:G values below 1.5, the remainder of results falling between 1.5 and 2.0. This would again indicate that cases of multiple sclerosis exhibit a pronounced disturbance in their protein metabolism although this is more clearly evident from the electrophoretic A:G values also shown in this table.

TABLE 4.—QUANTITATIVE PROTEIN FLOCCULATION DATA FOR SERA FROM MULTIPLE SCLEROSIS CASES

No. of Cases	*Total Serum Gamma Globulin gm. per 100 cc.*	*Globulin Clot-Gamma Globulin gm. per 100 cc.*	*Gamma Globulin Electrophoretic gm. per 100 cc.*	*Total Serum-Globulin Clot*	*Electrophoretic* $\frac{\text{Beta + Gamma}}{\text{Gamma}}$
	Mean S.D.	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*
Multiple Sclerosis (23)	1.74±.19	1.13±.19	1.1±.17	1.55±.19	2.21±.22
	Range	*Range*	*Range*	*Range*	*Range*
	1.37–2.10	0.92–1.44	0.7–1.5	1.20–1.86	1.94–3.05
Normal Sera (32)	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*
	1.54±.23	1.00±.13	1.0±.21	1.55±.12	1.9±.24
	Range	*Range*	*Range*	*Range*	*Range*
	1.09–2.00	0.72–1.25	0.8–1.2	1.40–1.83	1.5–2.2

TABLE 5.—PROTEIN FRACTIONS OBTAINED BY SALT FRACTIONATION OF SERA FROM MULTIPLE SCLEROSIS CASES

No. of Cases	*Total Protein gm. per 100 cc.*	*Albumin gm. per 100 cc.*	*Globulin gm. per 100 cc.*	*A/G Ratio*	*Electrophoretic A/G Ratio*
	Mean S.D.	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*	*Mean S.D.*
Multiple Sclerosis Sera (23)	7.28±.57	4.40±.32	2.88±.55	1.58±.35	.97±.14
	6.50–8.82	3.92–5.25	1.74–4.15	1.02–2.78	.72–1.23
	Range	*Range*	*Range*	*Range*	*Range*
Normal Sera* (36)	7.2±.35	5.2±.25	2.0±.27	2.6±.26	1.29±.16

* Data taken from paper by Gutman, A. B., *et al.*, J. Clin. Invest., **20**, 765, 1941.

The above mentioned protein findings were equally observed in patients who were either in the state of remission or who had an exacerbation of their condition. Studies are now in progress to evaluate the laboratory data in a larger number of such cases.

Summary and Conclusions. 1. The distribution of the electrophoretic and chemical protein fractions of the sera from 23 carefuly selected cases of multiple sclerosis has been studied.

2. The typical electrophoretic serum protein pattern found in about 90% of the multiple sclerosis cases is a significantly decreased albumin and A:G ratio, significantly increased alpha-2 and beta globulin fractions, a slightly elevated or normal gamma globulin fraction and a normal alpha-1 globulin fraction. In the remaining 10% of the multiple sclerosis cases, most but not all of these differences in the serum protein fractions are present.

3. The simple, qualitative or semi-quantitative, protein flocculation reactions may prove to be of some use in making a clinical diagnosis of multiple sclerosis when a more elaborate method of determining changes in serum proteins is unavailable. Of those tested, the highest percentage of positive tests (66%) was given by the cephalin-cholesterol flocculation reaction of Hanger[5] which probably reflects changes in the albumin components. The next highest number of positive results (29%) was given by the thymol turbidity reaction[18] which probably reflects changes in the beta globulin fraction. The zinc sulfate turbidity reaction[15] and the ammonium sulfate-sodium chloride turbidity reaction[7] probably reflect changes in the gamma globulin fraction and show only a small number of positive tests.

4. The quantitative cephalin-cholesterol reaction with temperature variation[27] was applied to the sera from the multiple sclerosis cases and abnormal protein patterns were found in about 88% of the cases. A serum protein pattern similar to those found in cases of infectious hepatitis, was noted in 44% of the cases, while another 44% showed protein patterns similar to those found in chronic hepatitis and only 12% showed normal serum protein patterns.

5. The quantitative protein flocculation-ninhydrin method for serum-gamma globulin[29,30] was applied to the sera from the 23 cases of multiple sclerosis. The globulin clot-gamma globulin value correlated closely with the electrophoretic gamma globulin. It is believed that such a procedure may be useful in distinguishing multiple sclerosis from other related diseases, for instance, post-encephalitic Parkinsonism, where the serum-gamma globulin values are usually high. The total serum-gamma globulin values were found to be significantly increased reflecting changes in the beta globulins, while the $\frac{\text{(total serum-gamma globulin)}}{\text{(globulin clot-gamma globulin)}}$ ratio is normal for this disease.

6. A sodium sulfite-salt fractionation-biuret method was applied to the sera from the 23 cases of multiple sclerosis. The total protein values were found to be normal but the albumin values were decreased and the total globulin values increased above that of normal sera resulting in a somewhat lowered A:G ratio.

7. These serum protein findings can be used to supplement already established cerebrospinal fluid procedures for purposes of diagnosis and possible prognosis of cases of multiple sclerosis.

Abnormalities in cerebrospinal fluid protein have been demonstrated by the immunochemical method for gamma globulin[10] and by the quantitatively standardized colloidal gold reaction[35] in 85 to 90% of a larger group of multiple sclerosis cases. Simplified procedures for determining *quantitatively* the gamma globulin content of cerebrospinal fluid employing protein floccula-

tion methods have been developed in this laboratory. Work is now in progress on the application of this procedure to cases of multiple sclerosis in conjunction with simultaneous studies of their serum protein fractions. It is planned to extend these studies to other diseases of the central nervous system including other related demyelinating diseases.

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ANTIBIOTIC-RESISTANT STAPHYLOCOCCI AND RELATED INFECTIONS

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FOR more than 5 years, observers in the fields of microbiology and clinical medicine have watched *Micrococcus pyogenes* acquire a new prominence in the medical literature. Most of the numerous articles have been stimulated by a growing concern over the behavior of this versatile micro-organism toward antibiotics in general, and penicillin in particular.

The intention of this review is to bring together opinions and conclusions representative of the literature and discuss various aspects of the problem now presented by antibiotic-resistant staphylococcal infections.

EFFECT OF PENICILLIN UPON MICROCOCCUS PYOGENES. The reaction of micro-organisms exposed to chemotherapeutic agents has been widely discussed. Garrod[46] has summarized the possible effects of these agents on bacteria as follows: first, they may be inhibitory or lethal; second, the micro-organism may be led to acquire resistance; third, dependence upon the drug may develop, and, lastly, the effect may be actual stimulation of bacterial growth. These categories make a convenient frame into which the behavior of staphylococci toward penicillin may be fitted.

The strain may be sensitive to low concentrations of the antibiotic. For practical purposes, such concentrations would be those attainable in body fluids, tissues, exudates, and inflammatory lesions. In the early days of penicillin therapy, most of the strains of staphylococci tested *in vitro* were found to belong to this category. From the beginning, the optimum effect of penicillin on the staphylococcus was believed to be bactericidal. This was Fleming's[42] original view, and it has been confirmed by Hobby *et al.*[59,60] and other investigators. In threshold concentrations, the drug is bacteriostatic.

There are a number of important aspects of this bactericidal effect *in vitro*, and these should be kept in mind by the clinician as well as the laboratory worker. In the first place, killing action is exerted on growing micro-organisms and is most marked upon young cells[17].

Second, the speed at which bactericidal effect takes place is relatively rapid. Exposure of penicillin-sensitive staphylococci to an *in vitro* concentration as low as 1.0 mcg. per cc. for one-half hour produces a decrease in the numbers of viable cells, while exposure of the micro-organisms to the same concentration for as short a period as 15 minutes results in an inhibition of growth for 2½ hours after the bacterial cells have been removed from contact with the penicillin[82,83]. If this effect is reproduced *in vivo* by the giving of the antibiotic before bacteriological procedures are completed, it explains some of the disappointments received from negative or insignificant culture results. Thirdly, with staphylococci, as with other Gram-positive cocci, it has been shown that the *in vitro* rate of destruction of bacteria follows increasing concentrations of penicillin until a maximum killing rate is attained, after which further increase in drug content has no observable effect[31,33]. The aim of therapy should, therefore, include an effort to achieve this optimum killing concentration at the site of infection. Lastly, changes in the morphology and staining characters of staphylococci occur, and have been described by numerous observers[29,45,51,67]. There is swelling of the bacterial cells and loss of Gram-positiveness followed by lysis. This rapid lysis of staphylococci in cultures was first reported by Fleming[42] and confirmed by others. The amount of penicillin necessary to produce this effect was found to be small rather than large. For example, staphylococci exposed to the low concentration of 0.1 mcg. per cc. were more rapidly and completely lysed than those exposed to as much as 100 mcg. per cc.[37,65,85].

There is a practical aspect to these facts also. Specimens, such as cerebrospinal fluid from a case of purulent meningitis, are often obtained from patients who have received what is aptly called "the parting shot" of penicillin before being sent into hospital. In giving an opinion on the probable etiology of the infection, the medical bacteriologist has to keep in mind that possible changes in morphology and Gram-staining may have already occurred in penicillin-sensitive bacteria.

Resistance to the antibiotic may develop. Early observations on chemotherapy indicated that *Micrococcus pyogenes* might prove troublesome in this respect. Even before penicillin was available for general use, it was apparent that sulphonamides were less effective against staphylococci than against other Gram-positive cocci. Moreover, evidence soon became available to show that resistance to these drugs could be induced *in vitro*[103], that such strains produced para-aminobenzoic acid, and that infections due to sulphonamide-resistant staphylococci were increasing in frequency[102]. It has been most unfortunate, though not surprising, that the staphylococcus with its capacity for taking part in a variety of severe infectious processes, should also prove itself in possession of great and natural ability to acquire high degrees of resistance to penicillin and other antibiotics.

The general agreement is that there are 2 kinds of resistance to penicillin in staphylococci: (a) One is that which can be induced artificially *in vitro*, and was first described by Abraham *et al.*[1] in 1941. Further studies by a number of other investigators have afforded considerable information about the character of this type of resistance. It appears to be temporary in duration, with a fairly rapid return to almost the previous state of sensitivity following transfers in penicillin-free medium[104]. Klimek *et al.*[67], in a study of developing resistance of staphylococci to both penicillin and streptomycin, noted that

morphological and Gram-staining changes occurred, the cocci becoming pleomorphic, rod-shaped and Gram-negative. Resistant variants may lose their ability to grow anaerobically, to ferment the usual sugars, with the exception of glucose, and to grow in 6.5% sodium chloride[11]. Similar observations on fermentative behavior had been commented upon by Segalove[95]. Examining the effect of penicillin on toxin formation by both types of penicillin-resistant staphylococci[95,96], it was found that in particular culture media, production of rabbit and sheep red blood cell hemolysins, lethal factor and dermonecrotoxin was inhibited by penicillin, while there was not any inhibition of enterotoxin formation. Preformed staphylococcal alpha hemolysin or lethal factor was not neutralized by the addition of penicillin. Others[13] have found that alpha hemolysin or lethal factor production was not prevented by the presence of penicillin in a concentration which just permitted growth to occur. (b) While these investigations were under way, it became evident that another kind of staphylococcal resistance to penicillin existed. This was first realized through clinical observations, which showed that some strains of staphylococci were insensitive to penicillin upon primary isolation, and, though many patients had already received treatment with the antibiotic, many others had not.

Kirby[64,66] was the first to demonstrate the presence of a powerful inactivator in extracts of 7 strains of penicillin-resistant staphylococci and its absence from 7 penicillin-sensitive strains. The work was confirmed by Spink and Ferris[100], and the name penicillinase was used by Bondi and Dietz[15] to describe this inactivator. As a result of later investigations by North and Christie[80], Blair *et al.*[13], Spink and Ferris[101], Gilson and Parker[49] and others, it became clear that a distinction existed between artificially induced and "natural" resistance. In strains made artificially resistant, this state is claimed to be temporary and accompanied by a loss of virulence, while in those naturally resistant by virtue of their ability to produce penicillinase, full virulence for experimental animals is retained. It appears that very little penicillinase is produced by penicillin-sensitive strains of *Micrococcus pyogenes*. In one series of 70 strains examined, those sensitive to less than 0.1 unit of penicillin per cc. produced no penicillinase, while those inhibited by concentrations of between 0.1 to 1.0 unit per cc. formed only small amounts of the enzyme extracellularly[50]. In many strains of staphylococci, the greater part of penicillinase formation is intracellular, but some strains may produce quite abundant amounts extracellularly[61,64]. Strains trained *in vitro* to penicillin resistance form no penicillinase[1,13,86,100], though it has been reported[11] that staphylococci which have become resistant to as much as 1.0 to 4.0 mg. per cc. of penicillin, will form penicillinase in the presence of the drug.

A discussion of the mechanism of action of penicillin and other chemotherapeutic agents upon the staphylococcus is not within the scope of this review. The subject has been fully dealt with by Florey *et al.*[43]. More pertinent is the mechanism whereby staphylococcal resistance may develop, for this has an important bearing upon the clinical use of antibiotics, since their value is closely allied to the numbers of resistant strains which develop and their prevalence in infectious processes.

The subject has been a widely controversial one. There is the possibility that increased resistance to penicillin can be the outcome of two entirely different mechanisms. One of these is the development of an ability to produce

penicillinase, and the other, that strains of staphylococci believed to have acquired resistance *in vivo* may really have arisen from naturally insensitive bacterial cells. The view held by Abraham *et al.* in 1946[2] was that development of increased resistance to penicillin is a direct result of exposure to the antibiotic and is an adaptive change both inheritable and permanent. That put forward by Demerec[20] suggested that antibiotic-resistant cells occur as rare spontaneous mutations in the course of bacterial multiplication and that they are present in the original colony, with the antibiotic exerting a selective action. Ordinarily, it is presumed, these first step mutants are overwhelmed in numbers by the penicillin-sensitive cells, but, during exposure to suitable concentrations of penicillin, these are inactivated or destroyed, leaving the resistant individuals to grow out. In a recent study on the distribution of resistance to several antibiotics amongst 6 different bacterial species, including *M. pyogenes,* Eagle *et al.*[32] have reviewed these controversial points and feel they are not mutually exclusive. Their own observations indicate that the number of bacterial cells capable of growing out to form colonies declines with the concentration of antibiotic. The resistant colonies and their progeny are regularly more resistant than the original one, and, in general, this resistance is related to the concentration in which the resistant colony emerged. For example, at low levels, 10% to 90% of the bacterial population grew out to form slightly more resistant individuals, while at higher levels, only a few appeared. There was no indication of a stepwise emergence, but rather an even falling off between low and high concentrations.

Possibilities for the development of increased resistance are then divisible into 2 types. In the first, it is postulated that, at low level concentrations, a large percentage of bacterial cells will grow out to form colonies of individuals which are slightly more resistant than the parent cells. The resistant offspring may be either rare spontaneous mutants appearing during bacterial multiplication in the presence of the antibiotic and overgrowing the sensitive bacterial cells, or the result of an adaptive process taking place in the presence of the antibiotic. The second possibility occurs at high, or substerilizing concentrations of the drug. Here, only rare individual cells grow out to form highly resistant colonies. These are considered to be either "spontaneous mutants present in the original inoculum, and which grow out selectively in the presence of the antibiotic" or "the result of an adaptive process in the presence of the drug". Whether these observations are accepted or those of Demerec, which postulate a stepwise emergence of resistant mutants, their application to the use of penicillin means that dosage should be high enough to prevent the survival of first step mutants, or of spontaneous mutants arising at low level concentrations of the antibiotic.

This opinion and experimental evidence may be kept in mind while considering an earlier discussion on the significance of penicillinase production by "naturally resistant" strains of staphylococci in which Barber[5] brings up some practical points pertaining to the use of penicillin in treatment of staphylococcal infections. The first question asked is whether the degree of resistance to penicillin reported for a given strain of staphylococcus may be high enough to prevent successful treatment. It is pointed out that the inoculum used in testing a culture is of great importance and must be taken into account when the result is read. This argument is based on the concep-

tion that a heavy inoculum *in vitro* might be compared with a heavy infection *in vivo*. Further consideration of the factor of inoculum size will be given later in reviewing laboratory sensitivity test methods. The conclusion is reached that penicillin treatment of a heavy infection by staphylococci highly resistant to penicillin, would be almost useless. Support is given to this by the work of Gilson and Parker[49] which shows that the amount of penicillinase produced by naturally resistant staphylococci may vary by a thousandfold. Resistance of an infection to treatment with penicillin may therefore be expected to vary according to the amount and rate of production of penicillinase by the infecting strain. It cannot be expected that those forming large amounts of the enzyme will be influenced by concentrations of penicillin attainable in the body. The second point queries whether the resistance of penicillinase-producing strains results entirely from their ability to inactivate penicillin, or whether they also possess a "residual resistance" (to penicillin) entirely separate from the capacity to destroy the drug. Barber feels that this is the case, and Spink[99] comments that, while penicillinase production is not claimed to be the only biochemical mechanism underlying resistance, at least penicillin-resistant strains isolated from human sources have all formed the enzyme[8,72,44].

The last point poses a question that needs considering in individual circumstances. Do staphylococci readily become resistant to penicillin *in vivo*, and if so, in what way does this resistance differ from the kind acquired *in vitro*? Barber's suggestion is that what appears to be an increase in penicillin-resistant staphylococci following or during penicillin therapy may really be the result of a selective process in which sensitive bacterial cells are destroyed, while resistant ones survive and proceed to multiply. Resistant and sensitive strains cultured from the same patient were usually found to be of two different bacteriophage types. No evidence of differences in cultural or biochemical character, nor of pathogenicity for rabbits, could be found to provide distinctions between penicillin-sensitive and insensitive strains.

It is apparent by now, through laboratory and clinical reports, that staphylococci can become resistant to antibiotics other than penicillin, although so far this has not become a problem of therapy to any great extent. As would be expected, the first reports were of staphylococci trained to be resistant to streptomycin *in vitro*, or already insusceptible when isolated from human sources. Those who are concerned with staphylococcal carrier rates have already found an increasing frequency of strains resistant not only to penicillin and streptomycin, but to other antibiotics as well[19,91,92,93]. Observations made *in vitro*, and on clinical infections have shown that staphylococci may acquire resistance to aureomycin and terramycin fairly readily. Staphylococci isolated from a variety of clinical infections for which some patients had received antibiotic and others had not, have been tested against several of these drugs, namely streptomycin, aureomycin, chloromycetin, terramycin, bacitracin and polymyxin B (aerosporin)[12,40,56,69,99] and strains resistant to each have been found, although in small numbers. The findings of Spink[99] may be quoted as being representative of others. He examined 104 strains of *M. pyogenes* and found over 50% to be penicillin resistant; 20 strains required at least 62.5 mcg. per cc. of streptomycin for inhibition, one was resistant to aureomycin, resistance apparently being acquired during therapy.

Although none of the patients had received terramycin, 5 of the strains were resistant to the drug. On a weight for weight basis, chloromycetin was less effective than aureomycin or terramycin, 2 strains being highly insusceptible, and 5 required 62.5 mcg. per cc. to inhibit growth. For practical purposes in these and other studies, "resistance" is taken to be at that concentration of the drug *in vitro* which is greater than any therapeutically attainable level. It is not uncommon to isolate strains of *M. pyogenes* which show *in vitro* resistance to more than one antibiotic from the first[19], but reports do not show that strains resistant to all the available antibiotics occur frequently.

Some attention should be given to the value of laboratory methods for testing bacterial sensitivity to antibiotics, because these provide the basis for data on the prevalence of penicillin-resistant staphylococci.

There are 3 main techniques, with variations, in common use: (1) the tube dilution method, (2) the antibiotic-impregnated blotting or filter-paper disk, and (3) the agar plate, in which the antibiotic is incorporated with the medium or in which a well or gutter is cut out and the space filled with fluid or agar containing measured quantities of the drug. In the same category is the cylinder containing antibiotic, placed on the surface of the agar plate after seeding with the test organism.

Each method has its advantages, but all carry with them a fairly high percentage of error and none affords accurate quantitative information on the degree of sensitivity of the bacteria tested. A number of factors, which are easily variable from test to test, influence the values obtained. Those are the size of the inoculum, the medium, the period of incubation and the choice of end-point.

In attempting the correlation of laboratory with clinical results, the most important single one of these factors is probably that of inoculum, particularly when staphylococci and penicillin are concerned[6]. Using a small inoculum of 1000 to 30,000 bacterial cells per cc. of a penicillinase-producing staphylococcus, Kirby[66] demonstrated that no viable micro-organisms could be recovered after incubation with 5 units of penicillin per cc. for 24 hours. However, a large inoculum of 10×10^6 bacterial cells per cc. allowed multiplication to take place with as much as 100 units per cc. Similar results with penicillinase-forming staphylococci were obtained by Gilson and Parker[49] who found that, when a large inoculum was used, these strains had an average resistance to penicillin 250 times that of those not producing penicillinase, but only 8 times greater if the inoculum was small.

Recent study of, and comment on, the use of laboratory tests for sensitivity, together with opinions of their value, is to be found in the work of Fairbrother *et al.*[38], Jackson and Finland[62] and Spaulding and Anderson[98]. In general, fairly uniform results can be obtained by any of these methods, but it is noted[98] that penicillin, unlike the "broad spectrum" antibiotics, is rapidly bactericidal, and the disk method indicates bacteriostatic, not bactericidal, concentrations. Readings obtained for aureomycin sensitivity vary more widely than do those for either streptomycin or terramycin and because the ease with which an antibiotic diffuses through the agar affects the size of the zone of inhibition, it follows that the drug allowing the widest zone formation is not necessarily the one to which the test organism is most sensitive. It is common knowledge that clinical results do not always parallel *in vitro* ones, and these laboratory test methods should be re-

garded only as a reasonable guide to the susceptibility or insusceptibility of a given bacterial strain to an antibiotic.

Evidence pointing to the development of dependence upon penicillin in staphylococci is lacking, but it has been shown to occur with streptomycin[111], and a theory as to the nature of the change has been suggested[81]. Very briefly, this is based on the assumption that streptomycin may be an analogue of a co-enzyme taking part in an essential metabolic process. In sensitive bacterial cells, this is assumed to be blocked by streptomycin. As a result of mutation, streptomycin becomes able to function as co-enzyme, and is henceforth necessary for growth.

Stimulation of bacterial growth may occur. Garrod[46] suggests that this type of bacterial reaction may be more common than is generally recognized, and points to the Arndt-Schulz law which states that poisons in small doses can act as stimulants. There is some supporting evidence quoted by Florey *et al.*[43] that, like other antibacterial agents such as sulphonamides, mercury compounds, arsenicals, phenols, and dyes, penicillin can exercise a stimulating effect on bacterial growth, and that this has been noted in staphylococci *in vitro*. By turbidity measurements and plate counts, it has been shown that the number of viable cells in a broth suspension of *M.pyogenes*, exposed to 0.01 unit per cc. of penicillin for 16 hours at 24°C, amounted to about twice the number compared to the same medium without penicillin. When the staphylococcus is used as a test organism in cylinder-plate assays of penicillin, an area of increased growth has sometimes been observed outside the zone of inhibition. By flooding the plates with redox indicators, Dufrenoy and Pratt[28] were able to show that the boundary zones exhibited a marked reducing power, and suggested this might be due to increased metabolic activity resulting from stimulation by sub-inhibitory amounts of penicillin. The possibility that bacterial growth might be stimulated *in vivo* through sub-inhibitory concentrations of antibiotic in the body during therapy, is an interesting conjecture. From the first, it has been the common experience to encounter cases where penicillin-sensitive Gram-positive bacteria have been replaced during therapy by penicillin-resistant Gram-negative ones. Inferences have been that the suppression of one type of bacterial flora leaves an area vacant for occupation by another type, which is insensitive to the suppressing agent. Garrod feels this view is justifiable only on the assumption that normal balance is maintained by antibiosis. It fails to explain many cases in which resistant bacteria appeared where none were before and might just as reasonably be attributed to a stimulative effect by the antibiotic. Towards support of this theory, he cites examples of 3 patients with subacute bacterial endocarditis who developed urinary tract infections with *Pseudomonas* while under penicillin therapy. This brings to mind more recent clinical reports where staphylococci are reported to have become predominant, replacing the original etiologic agents in the sputum of pneumonia patients[63], in feces[41,63,112] in the urinary tract[63,112], and as a cause of "staphylococcal scarlet fever"[41,55].

It has been suggested that reversion from penicillin-resistance to relative sensitivity might occur *in vivo*, but there is almost no supporting proof that it can. Voureka[107] described a series of *in vitro* experiments which claimed to show that penicillin-resistant staphylococci could become sensitive following contact with various other bacteria, among these being penicillin-sensitive streptococci. It was suggested that a similar change might occur *in vivo*, and aid in suppressing the tendency for antibiotic-resistant strains to develop.

These experiments have not received satisfactory confirmation, but merit further study, particularly since the work of Hotchkiss reported at the recent International Congress for Biochemistry[34]. Working with pneumococci, he has shown that penicillin resistance can be transferred in a fashion similar to that employed by Avery for the transformation of the capsular polysaccharides. By adding an extract of penicillin-resistant pneumococci to the medium used for growing penicillin-sensitive pneumococci, he obtained penicillin-resistant individuals from among the offspring. The resistance of these persisted in subcultures. The "transforming factor" is said to be a desoxyribonucleic acid, and is separate from the capsular polysaccharide factor. The inference, then, is that penicillin-resistance is determined by the desoxyribonucleic acid of the bacterial cell and can be transferred, thus permitting sensitive organisms to become resistant without ever being exposed to penicillin. It is tempting to speculate on the possibility of a similar factor existing among staphylococci and operating *in vivo*.

THE PREVALENCE OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCI. It has already been pointed out that naturally resistant strains of staphylococci were rarely encountered before penicillin became generally available for unrestricted use in therapy, after which it was only a short time before reports, noting the isolation of antibiotic-resistant strains from a variety of sources, began to appear. As might be expected, the earliest clinical observations came from military hospitals and wherever open wounds formed a large proportion of the cases being treated. This was soon followed by accounts of staphylococcal resistance to penicillin, apparently acquired during therapy and held responsible for a number of unsuccessful results[84,94]. In an early study of strains of staphylococcus isolated from 59 patients[80], it was found that only penicillin-sensitive cultures were recovered in 10 patients who did well under therapy. In 35 cases, only resistant strains were found at any time, while in 10 others, the penicillin-sensitive strains were replaced by resistant ones in the course of treatment. In 4, sensitive or resistant staphylococci were isolated alternatively. In another series[13], where staphylococci cultured from patients with osteomyelitis were examined for sensitivity to penicillin, it was noted that the great majority of strains which became resistant were those from surgical wounds healing by granulation or delayed closure, and very rarely after primary closure. Reports of this kind were not numerous enough to lead to any particular apprehension that antibiotic resistance among staphylococci might develop into a clinical problem. The significance of these and similar observations was first really appreciated through a series of publications by Barber and her colleagues[5-9] and Rountree *et al.*[89,91-92,93]. These studies were primarily concerned with the progressive increase in the number of penicillin-resistant staphylococci being isolated from patients, the mechanism by which these strains might have acquired resistance, and the correlated problem of cross-infection.

Some mention should be made of earlier surveys on the incidence of staphylococcal carriers. The interest which has lately been focussed on the detection of nasal carriers is not new, but has acquired added significance because of the problems connected with cross-infections in hospital communities where penicillin- and other antibiotic-resistant staphylococcal infections are most prevalent at the moment. In a report dealing with investigations into the possible relationship between nasal carriage and staphylo-

coccal wound infections, Miles *et al.*[76] have tabulated the findings of preceding authors. These show that the incidence of nasal carriers which has been obtained from several groups of 40 to 159 individuals each, ranged between 32% and 43%, with skin carriage in from 5% to 24% of persons. Their own study has added some interesting information about staphylococcal carriers. Results of nasal swabbings from 479 normal adults attending hospital for the first time showed that between 40% and 50% carried *M.pyogenes* in rela-

TABLE 1.—REPORTED INCIDENCE OF STAPHYLOCOCCAL CARRIERS, 1947–1952

Authors	*Numbers and Groups Cultured*	*Carrier Site*	*Incidence of M. pyogenes*	*% of Antibiotic-resistant Strains*
Allison, V. D., & Hobbs, 1947[3]	103 Hospital staff	Nose	70%	
Martin, T. D. M., & Whitehead, J. E. M. 1949[71]	50 Healthy adults	Nose, skin or throat	62%	19.4% (Penicillin)
Barber *et al.*, 1949[7]	62 Department store women	Nose	33%	0% (Penicillin)
Martyn, G., 1949[74]	130 Normal infants	Nasopharynx, Feces	62% 50%	55.5% 58.5% (Penicillin)
Rountree, P. M., & Thomson, E. F., 1949[92]	200 Hospital staff	Nose		32% (Penicillin)
	200 Blood donors	Nose		4% (Penicillin)
Denton, G. D., *et al.*, 1950[21]	375 *Maternity center:* Infants, mothers, doctors, nurses, laundry staff	Nose	240 (64.0%)	32.5% (Penicillin)
	484 *Controls: Not Mat. cent.* Grad. Sch. nurses, P.H. nurses, Student nurses, Science students, Phys. Ed. students, Internes	Nose	139 (28.7%)	
	50 *Some through Mat.* 4th year med. students	Nose	23 (46.0%)	
	50 *Not through Mat.* 2nd year med. students	Nose	20%	
Rountree, P. M., *et al.*, 1951[91]	513 In-patients	Nose		53.4% (Penicillin) 14.0% (Streptomycin) 47.0% (Pen. & Strep.)
	90 Out-patients	Nose		24.6% (Penicillin)
Wilson & Cockroft, 1952[110]	110 Children (6–16 yrs.) Out-patients	Nose	65%	11%
	40 City Health Dept. staff	Nose	27%	18% (Penicillin)
	68 Maternity Hosp. staff	Nose	48%	70% (Penicillin)
	68 O.R. staff	Nose	51%	79% (Penicillin)

tively large numbers in the anterior nares, and between 10% and 20% carried organisms on the back of the wrist as well. A marked correlation was found between phage types of staphylococci from these 2 sites in individuals[109]. It was also shown that a wide fluctuation in carrier incidence occurred over the 10-month period of the investigation, between October and July. In the study of infected wounds, it was of interest that no positive association between staphylococcal carriage in a patient's nose and the wound infecting strain could be established, and it was believed that this pointed to the prevalence of cross-infection from other sources, rather than to self-infection. The authors offered the opinion that the nasal carrier state varies, not according to a person's environment, but with the individual, and that there appears to be a distinct tendency for persons to be either persistent carriers of staphylococci or to be regularly free from them.

The foregoing table summarizes the results accumulated by various workers in several countries over the past 5 years. Their purpose is to show the comparative distribution of staphylococcus carriers in the population, and, to some extent, the prevalence of antibiotic-resistant strains among these.

From these figures, it is plain that staphylococcal nasal carriers are far more numerous in a hospital community than in the general public, and that the hospital carriers also show the highest percentages of penicillin-resistant strains. There does not appear to have been any marked increase in the incidence of carriers among the general population. Barber's reports, already referred to, noted over the years 1946 to 1948 a steep rise in the prevalence of penicillin-resistant staphylococci in cultures from the usual variety of infections seen in a large hospital, the figures being 14%, 38% and 59% in successive years. Additional inquiries were made into the types of resistant staphylococci, the role of penicillinase, its possible effects on the success of treatment, and the apparent development of resistant strains during penicillin therapy. Some of these points have been considered in the discussion of staphylococci "naturally" resistant through the ability to form penicillinase. With regard to the mechanism whereby penicillin-resistant staphylococci have increased and spread, Barber's opinion is both reasonable and well-supported by these early studies and also by later investigations. To recapitulate, her belief is that this emergence of progressively more numerous resistant strains has been brought about by a process of selection rather than mutation. Although it is probable that penicillinase-producing organisms were rare at first, the use of penicillin on such a wide scale has eliminated many of the sensitive ones, leaving the insensitive survivors to multiply, and these have been distributed and maintained in a locus largely by means of cross-infections. There is considerable supporting evidence for this view to be found in other studies on cross-infection[19,92,93].

Comparatively little attention has been paid to carriers who harbor coagulase-positive staphylococci in feces, which is far more frequent than is generally believed. It will be seen from the preceding table that Martyn[73] found coagulase-positive staphylococci in 50% of feces cultured from 130 babies under 1 year of age, while the incidence of nose or throat carriers in the group was 62%. Penicillin-resistant strains formed 55 to 58% of those isolated from both sources. This high carrier rate was revealed by the use of Robertson's meat medium containing 10% of sodium chloride, from which subcultures were made after overnight incubation. By a somewhat different technique, employing a

phenol red-mannitol agar containing 7.5% NaCl, staphylococci were recovered from the feces of adults in approximately 31% of cases[18], in numbers estimated as 1 to 62 millions per 100 gm. of feces. At this hospital, (Children's Memorial Hospital, Montreal, P.Q.) agar plates containing 7.5% NaCl have been included as a selective medium in addition to the usual media used for the isolation of Salmonella and Shigella. The results of this 3-year survey are given in tables.

The figures in Tables 2, 3 and 4 are found to be statistically significant; only those for the over 2 years group in Table 4 are pronounced border line. They show *M.pyogenes* is more common in the feces of children under 2 years of age than in older children. In the 1,663 patients considered the increase occurred abruptly in the younger children and gradually in the older children during the same period of years. Children receiving one or more of the available antibiotics for varying periods, showed a decreased incidence of staphylococcus in the stools of those under two and not in the older group. The figures raise the question whether the antibiotic has in some way stimulated an increase in incidence of staphylococcus in the stools of the older children.

TABLE 2.—INCIDENCE OF M. PYOGENES IN FECES

Age Group	*Total No. Patients*	*M. pyogenes Present*
Under 2 years	1173	537 (45.8%)
Over 2 years	490	138 (28.2%)
TOTAL	1663	675 (40.5%)

TABLE 3.—M. PYOGENES: ISOLATION FROM FECES OF 1663 PATIENTS IN TWO AGE GROUPS

OVER 2 YEARS

Year	*Number*	*M. pyogenes present*
1950	141	22 (15.5%)
1951	158	37 (23.4%)
1952	191	79 (41.3%)

UNDER 2 YEARS

Year	*Number*	*M. pyogenes present*
1950	409	152 (37.1%)
1951	392	196 (50.0%)
1952	372	189 (50.8%)

TABLE 4.—INCIDENCE OF M. PYOGENES IN FECES—IN RELATION TO ANTIBIOTIC THERAPY

Age Group	*On Antibiotic*		*No Antibiotic*	
Staph. Culture	+	−	+	−
Under 2 years	225 (37.3%)	378	312 (54.7%)	258
Over 2 years	62 (33.9%)	121	76 (24.7%)	231
TOTAL	287 (36.5%)	499	388 (43.5%)	489

The question of whether *Micrococcus pyogenes* can be held responsible for some cases of infantile diarrhea and vomiting has not yet received a conclusive answer, although investigations into this possibility have been made[27,74]. Regardless of this aspect of staphylococcal infection, it seems reasonable to suggest that the feces must be considered equal to the nasal carrier in importance as a source from which cross-infections may arise, particularly on infants' wards.

CLINICAL INFECTIONS WITH ANTIBIOTIC-RESISTANT STAPHYLOCOCCI. The increased prevalence of antibiotic-resistant staphylococci is an accepted situation, and a variety of infections caused by these strains is now being reported from many sources. Such infections can be just as diverse in character as those caused by strains which are sensitive to a wide range of antibiotics. Many have been severe, often following upon, or complicating, the use of penicillin or other antibiotics. Equally acute infections have occurred in patients known to have received no antibiotic therapy of any kind. Some have developed on the ward through cross-infection, others in persons who had never been in hospital before. Finland[41], in remarking upon the increasing importance of the clinical problem presented by these infections, speculates, as others have done[46], whether this might be, at least partly, the consequence of widespread antibiotic therapy and related to the changes which occur in bacterial flora during chemotherapy. He notes the varied circumstances under which the staphylococcus has manifested itself, and cites as examples its appearance in the sputum of patients being treated for other respiratory tract infections; in clinical "staphylococcal scarlet fever" following therapy for unrelated infections, and, as the predominating organism cultured from the feces containing mucus and pus or blood of some patients receiving terramycin or aureomycin. This led to the term "staphylococcal dysentery" being applied to such cases, in one of which the staphylococcus also invaded the blood stream giving rise to a bacteremia.

A number of other authors have contributed information on occurrences of a similar character, the following being representative of the type in which staphylococci have made their appearance and reached predominance in locations where they were absent or in the minority before therapy was instituted. In a series of 91 patients with pneumonia, for the greater part associated with *Diplococcus pneumoniae*, there were a number of serious complications in which treatment with terramycin was accompanied by the appearance of *M.pyogenes*. The most important feature was considered to be the rapidity and frequency with which staphylococci emerged and replaced other bacterial species in the sputum. The majority of strains were highly resistant to terramycin. In 4 patients, definite involvement of the lungs occurred seemingly as superinfections; 9 developed severe diarrhea and staphylococci were cultured in large numbers from the watery feces and proctoscopic examination revealed ulcerations of the mucosa in most of the cases; there was one instance of urinary tract involvement. Of the 7 fatal cases in this series, super-imposed staphylococcal infection was believed to have accounted for 4, which was confirmed at necropsy in 1 case[63].

Terramycin used in 112 adults with chronic urinary tract infections associated with *Escherichia coli* or *Aerobacter aerogenes* was considered to be responible for the frequency with which staphylococci appeared and persisted during therapy. Of particular note were 2 cases, one endocarditis with embolic phenomena and the other a

"staphylococcal dysentery". Several other patients acquired staphylococcal pneumonia[112].

In a comparative study of the effects of either penicillin, aureomycin, chloromycetin or terramycin on pertussis in 150 children, Hazen *et al.*[55] have also described 2 severe infections in which staphylococci were incriminated. In one child receiving terramycin, staphylococci increased in numbers in cultures from the upper respiratory tract, and, on the third day of treatment, a scarlatiniform rash appeared, followed by ulcerative stomatitis, pharyngitis and diarrhea with vomiting on the fifth day. The other patient developed the same signs and symptoms on the ninth day of aureomycin therapy.

Until more information became available about penicillinase and the possible mechanisms by which staphylococci might appear to acquire resistance, doubt existed as to whether the increased tolerance to the penicillin could develop *in vivo* during treatment of infectious processes where the possibility of cross-infection was most improbable. In 1946, Dowling *et al.*[26] described the case of a patient in which the strain of *M.pyogenes* became resistant to penicillin during treatment of a bacteremia, and this account was later amplified when each of the infecting strains of staphylococci from a total of 4 cases of bacteremia or endocarditis were reported to have become resistant in the course of therapy[58].

Levinson[68] has analysed some results of antibiotic treatment from a series of 46 cases of patients with bacterial endocarditis, 10 of which were due to coagulase-positive staphylococci. Four of the strains were initially sensitive to penicillin and appeared to remain so, but 1 strain, also initially sensitive, became resistant to 60 units per cc. inside of 2-weeks' time. Two patients were infected, apparently from the first, with penicillin-resistant staphylococci, but the strains were inhibited by aureomycin. The penicillin-resistant strain from another patient developed resistance to aureomycin by the 9th day of therapy, 40 mcg. being required to produce inhibition *in vitro*.

The staphylococcal pneumonias described in connection with the problems presented by antibiotic-resistant strains, would appear to have become more prevalent over the last year or two. Many are of the type just mentioned, where the infection becomes manifest during the administration of antibiotic therapy intended to suppress a different micro-organism. There is no particular reason to suppose that any improvement in the situation can be expected under the present circumstances of widespread chemotherapy, and the use of antitoxin seems desirable in these intractable severe cases.

One of the most severe forms of staphylococcal infection is the fulminating pneumonia seen in infants, and there is some suggestion that this, too, may have increased in frequency[22,47,52]. It is questionable if the change could be connected with the use of antibiotics, because staphylococcal pneumonia often occurs in epidemic form, and any apparent increase might very well be part of such an episode. In this respect, an interesting annotation on the possible association between influenza virus and bacteria has been made[4]. The role of bacteria as opportunist in influenzal pneumonia is seen as an important one, and experimental evidence provided by a number of workers is quoted in pointing out the frequency with which influenza virus has been recovered from patients with bacterial pneumonias, particularly from those caused by staphylococci. Speculation is offered as to the possible part played by staphylococcal spreading factors, or by some sort of synergistic relationship.

In the past 2 years, at the Children's Memorial Hospital, Montreal, more than the average number of cases of staphylococcal pneumonia have been encountered in children, the great majority of whom were under one year of age. Those who survived the first few days of illness, usually with complicating empyema, could be given a more hopeful prognosis. Of those with infections which terminated fatally, the majority died between 24 to 48 hours after the known onset of symptoms.

TABLE 5.—INCIDENCE OF PROVEN FATAL CASES OF STAPHYLOCOCCAL PNEUMONIA IN A 10-YEAR PERIOD FROM 1942 TO 1952

Year	*No. of Admissions*	*No. of Necropsies*	*No. of Staph. Pneumonias*	*Percentage*
1942	3359	106	3	2.8
1943	3854	94	3	3.2
1944	4127	68	0	0
1945	4044	117	2	1.7
1946	3982	122	2	1.6
1947	3820	145	2	1.4
1948	4425	111	2	1.8
1949	4097	101	3	2.9
1950	4119	124	4	3.2
1951	4271	126	16	11.9
1952 (Dec. 1)	4372	143	12	(8.4)

In 1951, 13 patients survived the infection, and 16 died; in 1952 up to December 1st, 36 children have been admitted with staphylococcal pneumonia and empyema, and 12 of the patients have died. It is entirely possible that other children were treated for pneumonia due to the staphylococcus but, because of its prevalence in the nasopharynx in the lower age group, our summary has been made only from those patients who developed an empyema from which *M.pyogenes* was recovered in culture. Of the 13 strains obtained from surviving patients in 1951 and tested for sensitivity to antibiotics, 11 were resistant to 10 units of penicillin from the first. In 1952, 15 out of the 24 strains isolated from successfully treated cases have been sensitive to penicillin. Some of the staphylococci have been phage typed, but no particular significance can be found in the results. In these 2 years, of 13 strains typed, 6 belonged to 42B, 5 to 47D, and 2 to 47C.

(These figures have been provided by the courtesy of Dr. F. W. Wiglesworth and Dr. A. J. dePape, Children's Memorial Hospital, Montreal)

The obvious feature in Table 5 is the sharp rise in the number of fatal cases in 1951, and continuing into 1952. It may or may not be worth mentioning that in 1951 there was an epidemic of influenza which affected Montreal.

That form of staphylococcal infection known as staphylococcal folliculitis, or pemphigus neonatorum, is quite common in maternity wards and in nurseries, where it may assume epidemic proportions[7,21,39,73,77,79,108]. When the infecting staphylococci show resistance to a number of antibiotics, and the carrier rate of these strains among hospital personnel is high, therapy and the prevention of further infections present a difficult problem for the clinician. Superficial skin lesions, beginning as a vesicle and becoming pustular, sometimes accompanied by conjunctivitis, make up the majority of cases. It is an error to consider the mildest of these infections as merely troublesome but not serious; there are accounts of sta-

phylococcal pneumonia developing as a sequence to such lesions[22,39] and other late complications are not uncommon.

Bacteriophage typing of staphylococci has proved to be of considerable value in the investigation into the original source of an outbreak of staphylococcal folliculitis[7,21,36,73,77,108] although it has often been impossible to establish this with certainty. It has also been of use in tracing suspected carriers of antibiotic-resistant staphylococci associated with cross-infections on hospital wards[9,93]. Some interesting information on the distribution of staphylococcal types has been collected in the course of these investigations, all of which noted the high incidence of infections with penicillin-resistant strains, and a correspondingly high carrier rate among the hospital staff.

The results of phage typing have shown that one type of staphylococcus tends to establish itself as the predominant strain in a community such as a hospital, to become responsible for the majority of cross-infections there, and, frequently, to develop high degrees of resistance to penicillin or other antibiotics. In various epidemics, however, no one particular phage type or group has been incriminated with sufficient frequency to allow its being considered significant. Wallmark and Laurell[108] examining 2346 strains, found that 50% belonged in one of 3 groups, 5B, 2A, and 12, and it is of interest that, from 6 different studies of staphylococcal phage type distribution, all authors report a different phage type distribution[7,21,36,77,89,108]. Nevertheless, Barber and Whitehead[9] feel it is remarkable that staphylococci belonging to the 6/47 group have been isolated in so many centers, where they form a high proportion of the penicillin-resistant strains. Lowbury *et al.*[70], using aureomycin in a study of the control of colonization of burns by various microorganisms, attempted to show a relationship between resistance or sensitivity of staphylococci to that antibiotic and their sensitivity to certain phage types. They were able to obtain some evidence that there might be a degree of correlation between the two, with certain types notably 76 and 31B forming a large number of the strains resistant to aureomycin. Staphylococci of type 7 and 75A tended to be sensitive strains.

A remarkable and thought-provoking study on the subject of "bacteriophage as a factor in epidemiology and bacterial evolution" has recently been published by Hewitt[57]. He presents experimental evidence to prove that staphylococcal phages can be adapted to lyse diphtheria bacilli, and suggests that this provides a reasonable explanation for the wide varieties of diphtheria strains found in infections, since staphylococci are so prevalent in the nasopharynx. He feels that this phenomenon may not be confined to these particular phages and diphtheria bacilli, but rather that "through the intervention of bacteriophages from phage-carrying strains, coincident infection with different bacteria may modify the character of many other pathogenic organisms. Indeed, the apparently inexplicable rise and fall in the severity of epidemics may be explained, at least in part, by the selective action of bacteriophage". It is suggested that in the intestinal tract, the "complex interplay of phage-carrying organisms and susceptible strains would result in modification of the flora by the selection of resistant mutants; and this may account for the wide variations seen, for example, in the Salmonella group". The author points out that it "seems probable that phages are important agents in the modification of infections and epidemics, and in the evolution of bacteria".

Perhaps this concept should be related to the work of Lederberg and

his colleague on "genetic exchange" in bacteria.

THE PREVENTION AND CONTROL OF STAPHYLOCOCCAL INFECTIONS. Fundamentally, no difference has been found in the methods for prevention or control of staphylococcal infections once the division of antibiotic-resistant from antibiotic-sensitive strains has been made. The majority of mild and moderately severe clinical infections with strains sensitive to one or more chemotherapeutic agents will usually respond quite readily to therapy with the appropriate drug, provided a bactericidal or inhibitory concentration can be attained in the immediate vicinity of the infecting organisms. It has been seen that infections with staphylococci resistant to therapeutically attainable concentrations of one or other antibiotics can present a serious problem in clinical practice, and it has been alarming to find reports of, and enounter, increasing numbers of these strains that exhibit "multiple" or "complete" resistance. The question follows as to whether the widespread and often indiscriminate use of antibiotics has contributed to produce this situation, and many are of the opinion that it has. If so, then it is likely that the proportion of staphylococci resistant to all antibiotics will continue to increase. This leads one to speculate on whether restriction on the use of these drugs to those with infections urgently requiring them, would do more than retard the process.

There is a general conviction that the increasing prevalence of these drug-resistant strains of staphylococci is largely the outcome of cross infections from carriers and clinically infected persons among the hospital personnel and patients. The organisms may be isolated from their nose, or throat, skin, and feces, and have been recovered from dust, air and bedding on wards where the infections have occurred, and spread to new hosts can take place by a variety of routes. It is obviously impossible to isolate all carriers; nevertheless, opportunities for cross-infection can be cut down or prevented if the simple fact is realized that hands appear to be the chief means by which bacteria from the nose and throat are distributed. The most frequent of these is by transfer of droplets sprayed during coughing or sneezing into the fingers or a carelessly used handkerchief [30,53,54,76]. It is felt that the conscientious use of strict precautions and isolation technique can be of great value in combatting the cross-infections which are most dangerous on infant wards.

The Medical Research Council Memorandum[75] on the control of cross-infections in hospitals affords a thorough and practical study of the subject. Advocating the importance of factors such as properly constructed buildings, adequate equipment, space and ventilation, it goes on to lay stress on the 3 things considered to be essential for the control of cross-infection. These are adequate isolation facilities, adequate sterilizing and disinfection equipment, and adequate staff who have been trained to maintain strict isolation technique. The Report provides a discussion of the prevalence and consequences of cross-infection, followed by a section in which are listed the possible sources and modes of infection. Particularly useful are the suggestions made for the prevention of spread and for control. These contain practical and detailed recommendations on isolation techniques in an outbreak of pemphigus neonatorum, control of droplet-borne infection, the laying of dust on floors and from bedding and the use of disinfectants in a variety of circumstances. Care of the newborn receives special consideration, and the report condemns the practice of placing infants in large communal nurseries where infection may spread with alarm-

ing rapidity, rather than in small units of 2 to 6 beds.

When severe infections with antibiotic-resistant staphylococci occur, consideration may well be given to the use of antitoxin. With the arrival of penicillin and, later, the "broad spectrum" antibiotics, the immunological approach to treatment was almost entirely put aside in favour of chemotherapy, although there has never been any evidence that the array of toxic substances which the staphylococcus is capable of producing are neutralized or diretly interfered with by antibiotics *in vivo*. It is worth while to recall the variety of known staphylococcus toxins and enzymes, 3 or 4 of which are hemolytic toxins, including the best known "alpha hemolysin" which is also associated with dermonecrotic and lethal actions. Evidence that these toxins are produced *in vivo* has been presented by Dolman[23]. Others which have been recognized are enterotoxin, at least two leukocidins, fibrinolysin, coagulase, hyaluronidase, protease and lipase. Relatively little is known about the modes of action of these substances[106].

Reports on the results obtained by the use of staphylococcal antitoxin and toxoid in the treatment of a large series of patients suffering from a wide variety of staphylococcal infections have been published in a number of papers by Dolman[23,24,25], who has been mainly responsible for the introduction of this form of therapy into Canada. The properties of staphylococcus antitoxin have been found to include the power to neutralize the hemolysins and the pathogenic effects of the lethal and dermonecrotic factors in the cells and tissues of laboratory animals, including the leukocidins. It is the view of Valentine[105] that the value of anti-leukocidin may well outweigh that of hemolysin neutralization in the prevention of further invasion of the tissues before the body has had time to produce its own antibodies in acute infections. In reporting on the clinical effects obtained in the treatment of 104 patients, which proved to be most encouraging, the limitations of antitoxic therapy are pointed out, and the necessity for early diagnosis and institution of antitoxic-serum therapy are emphasized. It is advocated that antitoxin be given in the treatment of acute and generalized staphylococcal infections, but it is not recommended for the obviously moribund patient. The tendency for staphylococcal infections, particularly furunculosis, to recur and become chronic, even after apparently successful antibiotic therapy, leads to the recommendation that staphylococcus toxoid be used for the prevention of recurrent infections[25].

Two of the more interesting recent contributions to our knowledge of immunity in staphylococcal infections, and which might prove to have a practical aspect in their control, have been made by Murray[78] and Rountree[90]. The former notes that investigations have demonstrated a transmission of staphylococcal antitoxin from mother to fetus, and that there is a relationship between the antibody level of mother and child. In most cases, the antibody level is higher in the infant, and in the cord blood, by 63%. It might not be wholly impossible to use this evidence as the basis for experimental immunization of mothers whose child is expected to be born at a maternity unit where staphylococcal folliculitis is endemic. Rountree notes that the greater part of studies on immunity in connection with staphylococci have been concerned with the antitoxic properties. Her feeling is that little convincing evidence exists to prove that the presence of circulating antitoxin "has any substantial effect on the course of staphylococcal infection in man", and that this is especially true of the newborn who, in spite of detectable circulating

antitoxin acquired by placental transfer, are particularly susceptible to fulminating infections quite different, clinically, from the infectious processes seen in adults, in whom hypersensitivity may play an important role.

Following up other workers' descriptions of an erythrocyte-coating polysaccharide antigen in staphylococci, it was conceived possible that it might be used to measure antibody levels in human sera, especially in the investigation of antibacterial antibodies. The results of such studies indicate that the antibody to erythrocyte-coating polysaccharide is absent from the majority of sera from infants of the newborn period up to 4 months of age. The antibody apparently does not pass the placental barrier, even though present in large amounts in the mother's serum. This is contrasted with Murray's[78] findings on anti-alpha hemolysin placental transmissions. Rountree finds there is no direct evidence that the absence of antibody to the erythrocyte-coating polysaccharide determines the differences between the character of staphylococcal infections in adults and infants, but finds a correlation between absence of the antibody in infants and their susceptibility to nasal colonization by staphylococci.

Staphylococcal coagulases have been investigated by Rammelkamp *et al.*[87] who reviewed previous work and contributed new information to the subject. The antigenicity of cell-free coagulase has been demonstrated by the production of a coagulase inhibitor, which is believed to be an antibody. The results indicate that there are at least 2 separate coagulases, produced by different strains of staphylococci. Nevertheless it is claimed from the clinical aspect that low titers of coagulase inhibitor, or non-inhibitory sera, are found more frequently in patients suffering from major staphylococcal infection than in healthy persons. The suggestion is made that a low anticoagulase titer predisposes an individual to infection with these microorganisms.

Elek and Levy[35] place paper strips saturated with staphylococcal antitoxin on the surface of a blood agar plate and the test strain is then streaked at right angles; the toxins diffusing from the growing bacteria produce an observable line, or pattern, of toxin-antitoxin flocculation. In spite of the acknowledged limitations of this method, it is felt that a good correlation exists between the production of diffusible antigens and other criteria of pathogenicity. By analogy with the Clostridia, it is suggested that the character of a staphylococcal infection may, in part, be determined by the toxic pattern and that this might afford one explanation of why some strains of *M.pyogenes* produce comparatively superficial infections, while others are markedly invasive.

Sherris and Florey[97] have discussed the possible relationship of penicillin sensitivity or resistance of strains to the types of clinical infections encountered; and with this in mind they have analysed a group of patients suffering from a variety of staphylococcal infections. As a result, they have been led to believe that penicillin-sensitive strains generally tended to be associated with lesions which were closed or deep-seated, regardless of whether the process was an acute or chronic one, while the resistant strains were usually associated with superficial infections. The implication is that, from a clinical viewpoint, many of the penicillin-resistant strains of staphylococci being reported from all sources, may really be less significant in the pathogenesis of severe infection than those which are sensitive to penicillin. A similar type of analysis by Reiss[88] failed to confirm these findings, and concluded that the sensitivity or resistance of a strain of

M.pyogenes cannot be correlated with the nature or severity of the infection process; which agrees with our personal experience.

Gillespie and Alder[48] have observed a lipase activity in many coagulase-positive staphylococcus cultures, absent in all coagulase-negative cultures, which is neutralized by antitoxin serum. It resembles the lecithovitellin reaction only superficially. Most of the penicillin-resistant strains are negative to this reaction and are most prevalent (82%) in cultures from out-patients as compared with cultures from in-patients (45%) and hospital staff. These associations are striking though not absolute and deserve further investigation.

Summary. The subject of antibiotic-resistant staphylococci and related infections has been reviewed from representative medical literature.

This survey draws attention to the possible ways in which *Micrococcus pyogenes* may respond to exposure to antibiotics, particularly to penicillin, together with their possible bearing upon therapy. It is also noted that strains of staphylococcus are becoming resistant to other antibiotics, although at a slower rate. Consideration is then given to the prevalence of antibiotic-resistant staphylococci among the population, and their comparative frequencies in healthy persons, hospital staff and infant and adult patients. The means of spread of these resistant strains is linked to cross-infection, and general measures to assist in its control are pointed out.

An attempt has been made to indicate the influence of the widespread use of antibiotics on the character of staphylococcus infections, and the clinical and bacteriological problems involved.

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ERRATUM

In the November 1952 issue, page 573, the first line reads "Dr. George Hunter published"— Should read "Dr. George Huntington".

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SYNERGISM AND ANTAGONISM IN MASS DISEASE OF MAN

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THAT one disease may change the course of another is an old, a familiar, and a poorly understood observation. The end result is also commonly different. Much depends upon the nature of the two diseases and the order in which they appear in the single host involved. The outcome expressed in terms of death, recovery or permanent defect theoretically is cumulative but something less than the sum of results of diseases acting independently.

The effect observed in nature is subject to wide variation from calculated values, whether the unit of observation is the single patient and the method clinical, or is a population of people and mass disease examined epidemiologically. Two diseases sometimes have no discernible effect on each other. Other combinations lead to a result in excess of the calculated result of their common presence, as expressed in deaths, complications and days of disability. A third result is that a disease may exert a favorable or limiting effect on the disease already present, or that the second disease is itself restricted in its usual activities by presence of the first.

An attempt at analysis and interpretation of the effect of coexistent disease cuts across all fields of medicine and public health, for the phenomenon is general. Existing observations are scattered and for the most part clinical. The present purpose is to examine the interaction of multiple diseases in a single host, viewing the phenomenon as an independent or single biological process and to search for principle and mechanisms that determine relationships observed. The main interest is in the mass phenomenon, the manner and extent that one disease of a population affects the behavior of a second. Most of the evidence of necessity is from clinical and experimental sources, for the individual has had more attention than the community. The problem is

indeed as much epidemiological as clinical.

Co-existent disease in man is a problem of all regions and all social and cultural situations. The altered behavior when two diseases act together is demonstrated most dramatically and most clearly with the acute specific infections. The present interest originated, in fact, with the experience of Gordon [65b] a quarter century ago when infectious diseases were much more common and their effect more marked than now. An appreciable series of patients with both diphtheria and scarlet fever gave numbers of deaths materially greater than expected from the combined rates of the two diseases. Complications were in excess, and the period of disability much enhanced. Other combinations followed the same pattern, some in greater degree than others. Such instances of double infection in the United States and western Europe are fewer now because many of the infections are themselves less frequent. In less healthy areas of the world and particularly in the tropics the natural heritage of many individuals is an episode of two or even more co-existent infections sometime during life. The acute nature and short course of most specific infectious diseases tend to limit such events; undue prevalence of chronic infections as malaria and amebiasis favors their occurrence. The main problem in the United States and similar countries is with associations of an infection and a disease of some other class, or of a combination of two morbid conditions of non-infective nature.

The changing character of mass disease of human populations in the western world and to a lesser extent in other countries[50], whereby the leading causes of death are non-infective rather than communicable processes[48], has the result of adding significances to associated diseases and their effect one on the other. Heart disease, cancer and diabetes are chronic conditions. Their long duration increases the chance of intercurrent infection, or of another disease being superimposed upon the first. The identification of common associations, the action of one disease in engendering resistance or susceptibility to others, and the biological interaction of the two to produce an altered clinical course and result[65c] become matters of increasing moment in practical control and management, both of disease of the individual and in mass relationships where populations are the unit of observation.

The ability of man or any other host to resist disease depends on anatomic structure of the organism, on physiological functioning, and perhaps more than anything else on biochemical activities. An individual starts life successfully equipped to meet some diseases and inherently susceptible to others, a situation expressed by the reciprocal terms of innate resistance and susceptibility. As life proceeds, modifications in the processes of defense appear, sometimes associated with growth and development and sometimes acquired through contact with the environment to which the host is exposed.

Attention to acquired resistance has long focused on specific immunity because of the dominant position of the infections. Appreciation grows that many other mechanisms are involved in acquired body defense. Even with the infections[143], the total content of acquired resistance is more than the immunity derived through antibodies. The manner and extent to which an existing disease can alter host structure or function and thereby accentuate or neutralize the effects of a second disease, is one other feature of acquired resistance and susceptibility. The two opposite modes of action are known as synergism and antagonism.

With the direct purpose of limiting the problem at hand, co-existent disease is understood to be an association of two or more independent disease entities concurrently active in a single host, including the variations that arise through simultaneous initiation, through differences in order of appearance, and by variations in interval between onset. Instances of multiple disease where one process has ended before the other begins are excluded. A relation may well exist through inapparent or unappreciated residua. The cumulative effects of successive experiences with varied agents of disease are a considerable contribution to the wear and tear of life involved in the aging process, and presumably in the state of resistance and susceptibility. Also excluded are instances in which one condition is clearly a complication of the original process. The association of hypertension and cerebral hemorrhage is an example.

Synergism, generally defined, is the cooperative action of discrete agencies such that the total effect is greater than the sum of the two effects taken independently. Antagonism is the opposite. As applied to disease they represent an enlargement of the fundamental biologic phenomena of symbiosis and antibiosis, where symbiosis[2] is understood to denote the remarkable cooperative pairings of species, and antibiosis the opposite relationship of mutual antagonism. Thus a host species, and man is the principle concern, when invaded by an agent of disease either reacts to its own advantage through establishing symbiosis, or to its disadvantage through antibiosis. The matter of synergism and antagonism has to do with two or more agents of disease, or of disease processes when agents are unrecognized, and their effects on a designated host; a system then of at least three interacting elements, two agents of disease and a host, with more than two agents possible. Mechanisms conceivably include mutual interaction of the agents one on the other and subsequently of the complex on the host; or of one agent so modifying the host that it reacts atypically to the second. As defined, synergism and antagonism fall on a positive-negative scale, with a scatter of observations determined by differences in ecologic balance of host and agent in specific instances. The zero point separating the two is termed asynergy.

Altered Host Constitution and Reaction to Disease. A primary separation of two main categories of synergism and antagonism in human pathology is believed an aid in orderly examination of the phenomenon. In one instance, a disease takes effect in a host where body constitution has been altered by an existing morbid condition of some duration. The second category is where two or more disease agents or disease processes initiate activities in an essentially normal host at more or less the same time. Any observed effect arising from a common association would be expected in the first instance to relate most frequently to acquired resistance or susceptibility engendered by the primary process, with host factor the determining issue. Mechanisms in the second class presumably are varied and numerous; sometimes related to a biologic interaction of the two agents, of an agent and an environmental factor, or again through acquired body resistance or susceptibility.

CONGENITAL ANOMALIES. Congenital and developmental abnormalities are among the more evident morbid processes favoring occurrence of another disease. Inapparent structural anomalies of groups of cells, variations in the arrangement of functional parts of an organ or minor abnormalities of cellular metabolism or enzymic function also suffice to change the resistance

of a host, sometimes to the extent of determining activity of disease agents ordinarily incapable of disease production. Examples of hereditary or developmental abnormalities found in association with particular disease conditions are presented in Table 1, as an illustration of the kind of search, within major classes of disease, necessary to establish those relationships, in this instance limited to synergism. The list could be greatly extended.

ENDOCRINE DISORDERS. The homeostatic mechanisms by which the body compensates for deficiency or excess production of one hormone by adjusting the production of others, are outside present interests, although representative of the phenomenon at hand. Numerous observations have been made, however, on associations of endocrine and metabolic states with various independent diseases, Table 2. In contrast to congenital malformations, where the result of association with another disease is regularly synergistic, these diseases are characterized by a selective action, sometimes synergistic, in others antagonistic.

TABLE 1.—SYNERGISTIC ACTIVITY OF CONGENITAL ANOMALIES

Congenital Defect	*Associated Disease*	*Reference No.*
Pancreatic fibrosis with pulmonary cystic disease	Bronchiectasis and pulmonary infections	169
Congenital heart disease, patent ductus arteriosus	Subacute bacterial endocarditis	75
Tracheo-esophageal fistula	Pneumonia	114
Cleft palate	Sinusitis	62
Genito-urinary anomalies	Infections	82
CNS anomalies, meningocele	Meningitis	50
Mongolism	Infections, especially tuberculosis	19
Hirschprung's disease	Nutritional deficiencies	73
Dental defects of congenital syphilis	Caries	96
Agammaglobulinemia	Infections	30

Congenital defects often deprive the body of naturally existing protective barriers and favor entrance and activity of a variety of disease agents. Anomalies along the course of the urinary tract, from the kidney to the urethral meatus, in the form of horseshoe kidney, hydronephrosis, hydroureter, anomalous renal vessels, fistulae of the bladder, urethral strictures and penile deformities are clinically demonstrated[82] to predispose to bacterial infection. Patent ductus arteriosus, bicuspid aortic valves and septal defects[75] are commonly associated with subacute bacterial endocarditis. Pneumonia with tracheo-esophageal fistula[114], and meningitis in patients with meningocele[150], are even more clear-cut illustrations of disease relationships dependent upon anatomic defect.

The synergistic relation of diabetes mellitus to pyogenic infection is well established, in some instances attributable to deficient blood supply due to arteriosclerosis, in others to a favoring action of hyperglycemia on bacterial multiplication[131]. Tuberculosis is also frequently associated with diabetes, attack rates for tuberculosis among 3000 diabetics in Philadelphia[26] being twice the observed frequency in controls. Active tuberculosis was most frequent among young diabetics, those on larger doses of insulin and patients below standard weight. Explanation seemingly rests[155] in defective antibody for-

mation and decreased activity of phagocytes because of high glycogen content.

Cancer of the pancreas has been found[195] to be 4 to 8 times more common among diabetics than in the general population. Since diabetes usually precedes clinical evidence of neoplasm by several years, malignant destruction of the islets of Langerhans scarcely stands in casual relation. An opposite or antagonistic action of diabetes is based on the rarity of clinical observation of that disease in association with rheumatoid arthritis[1], asthma[187] and peptic ulcer[160], diseases in which Abrahamson[1] found by glucose tolerance studies a frequent occurrence of hyperinsulinism or dysinsulinism. The lack of association with peptic ulcer[160] has been attributed to diminution of gastric secretion in diabetes and masking of symptoms when they do occur.

Obesity is generally recognized[15,194] to have a synergistic relationship with both diabetes and hypertension. The 3 conditions occur together so consistently that Moss[139] in searching for factors contributing to endometrial adenocarcinoma was unable to distinguish between frequencies of the 3. Some 90% of 2,500 patients[15] developed definite obesity just prior to the appearance of diabetes.

TABLE 2.—RECORDED INSTANCES OF SYNERGISM AND ANTAGONISM BETWEEN HORMONAL AND METABOLIC CONDITIONS AND VARIOUS DISEASES

Condition	*Synergism*	*Reference No.*	*Antagonism*	*Reference No.*
Diabetes	Pyogenic infections	131	Rheumatic fever	195
	Tuberculosis	26	Rheumatoid arthritis	1
	Carcinoma of pancreas	195	Asthma	187
			Peptic ulcer	160
Hyperthyroidism	P.V.M. (mice)	201	Poliomyelitis (mice)	91
Hypothyroidism	Infections	74	P.V.M. (mice)	201
	Poliomyelitis (mice)	91		
Hibernation			Bacterial and Protozoal infections of animals	23, 99
Pituitary growth hormone	Influenza	105		
Testosterone	Influenza	105	Mouse leukemia	140
			Fowl lymphomatosis	31
Pregnancy	Poliomyelitis	10	Addison's disease	83
	Infective hepatitis	53	Myasthenia gravis	83
	Tuberculosis	155		
ACTH and cortisone	Plasmodia of Avian malaria	153		
	Pyogenic cocci	106		
	Tubercle bacillus	40		
	Vaccinia virus	112		
	Trichophyton	112		
	Trypanosoma cruzi	112		
	Poliomyelitis virus (mice)	166		
	Coxsackie virus (mice)	111		
	Encephalitis viruses	173		
Pregnancy, ACTH, cortisone, and jaundice			Rheumatoid arthritis	83
			Fibrositis	83
			Asthma	83
			Migraine	83
			Hay fever	83
			Psoriasis	83
Obesity	Diabetes	15		
	Hypertension	194		
Hypoparathyroidism	Moniliasis	180		

A strong clinical impression exists that the state of thyroid activity is related to susceptibility to infection, the decreased metabolism of hypothyroidism lowering resistance to bacterial infections such as tuberculosis[74]. Experimental support is had in the work of Holtman[91]. Normal mice inoculated intracerebrally with poliomyelitis virus had an average survival time of 7 days; administration of thiouracil, decreasing thyroid activity, reduced the time to 5 days; and supplementary thyroid substance led to increased survival, to 11 to 14 days. The opposite effect has been seen with other viruses[20], pneumonia virus of mice PVM; again no effect, Gallan[59] with MM virus and mice. Repeated experience of similar mechanisms acting on different agents, particularly viruses, to produce a different result suggests differences in metabolic requirements of the pathogens[105] as the determining factor.

Widespread studies[42] following the development of cortisone and ACTH have enlarged information on the relation of adrenal hormones and adrenal function to host resistance. Many infections are enhanced in pathogenicity, bacterial[106] including tuberculosis[40], protozoal[112,153] and viral[111,112,166,173], following administration of these hormones.

MENTAL DISORDERS. Less factual information probably exists on the influence of mental illness on physical disorder, or of one mental disorder on another, than for any other major class of human disease[67]. Deficiencies in clinical understanding are frequent, and in the epidemiological sense there is almost no information. General clinical belief relates psychic maladjustment to such somatic disorders as peptic ulcer, mucous colitis, hypertension, and rheumatoid arthritis, but proof is strikingly scant.

Patients in mental institutions commonly show peculiar associations of disease[107]. Tuberculosis[155], for instance, is much more frequent than in general populations, but whether attributable to the peculiarities of the environment, to the effect of specific mental disorder in an altered host constitution or to interrelated pathologic physiology is essentially uninvestigated. Angina and coronary thrombosis are 14 times more frequent in general populations than among mental patients; and peptic ulcer 3 times[72]. Such observations suggest fundamental differences in physical constitution, a more favorable environment, perhaps evidenced through relief of the ordinary stress and responsibility to which man is subject, or a possible antagonism. Few community surveys of mental illness have been made[159] and essentially no attempt to relate these mass mental problems to other and physical illnesses of a population.

NUTRITIONAL DISORDERS. The words famine, plague and pestilence have been traditionally linked throughout history. The implied relationship between disease and nutritional state has been accepted more because of reiteration[12] than because of evidence[165a]; and even more perhaps because that view is so reasonable. Sigerist[170] has suggested that other social dislocations accompanying famine are often more directly responsible for the spread of disease than is lack of food. Only recently has an old observation[186] received experimental support[36], that a nutritional deficiency, under certain circumstances, may be more harmful to an infectious agent than to the host, thus promoting antagonism rather than synergism.

The last decade has shown measurable progress in evaluating the confusing relationships between nutritional needs of disease agents and of the hosts they invade. Lack of some nutrients affects the host before the agent[109] with the result that host resistance is lowered. In other instances, the agent is the more susceptible[36] and the balance

turns in favor of the host. Studies on malnutrition and starvation in the course of the past war have led to general acceptance that although the incidence of certain infections does characteristically rise, tuberculosis being one[155], undernourishment does not necessarily lower resistance to all diseases. Gottlieb[69] reported a low incidence of infection among American prisoners of war. Keys *et al.*[109] in their studies on starvation in man found no increased incidence of infections, especially those of the upper respiratory tract.

The case for nutritional disease favoring the course of infection rests partly on the ability of protein starvation to inhibit production of antibodies. Certain specific vitamin deficiencies[9,177] have evidenced that capacity; and other reports[43] suggest that the same mechanism depresses phagocytic activity. In a series of studies, Cannon[34a,b] demonstrated that protein depletion interferes with antibody production; for a variety of antigens[203] and in several species of experimental animals, Schneider[165c] has made the point that the effects noted occur only in the presence of intense and prolonged protein deficiency, of a degree uncommon in nature. Animals subjected to less severe protein deficiencies[136], judged comparable to those of human populations[165c] reacted normally to antigenic stimulus[177]. Cachectic patients[14] suffering from gross protein deficiency had unimpaired immune body response.

Many infections have been studied in relation to the nutritional state of the host. Observations date from muscardine, a fungus infection of silk worms that provided one of the earliest demonstrations of an infectious agent. Bassi[16] remarks in 1836 that "good nutrition, complete health and vitality expose the silk worm more to infection than the opposite properties," and that "in a colony the sick worms always apparently appear to be the best."

Most helminth infections seem to be aggravated by nutritional deficiencies[145], but with protozoa the deficiency may act either in favor of the parasite or of the host[36]. Low levels of vitamin B complex in the pigeon have the effect of lowering resistance to trypanosome infection, in the rat resistance is increased. Studies of Faust and Kagy[52] led to the conclusion that transition from the carrier state in amebic infection to active amebiasis is dependent on nutrition of both the host and the parasite.

Interaction of nutritional disorders and bacterial infection has been investigated clinically, through laboratory experiment and by epidemiological methods. A clinical relationship between tuberculosis and the nutritional state of the host has long been accepted[155] as contributing to increased activity of the process. Epidemiological studies in Denmark[51] during World War I showed correlation between dietary intake and incidence of tuberculosis, an experience that was repeated in Germany[123] in the war that followed. Further proof has been contributed by laboratory study of mice[148] deficient in several nutrients including protein, thiamin and riboflavin, all of which contributed to a similarly increased susceptibility.

The methods of experimental epidemiology, using colonies of mice or other small animals have also been used in study of nutrition and resistance to infection. An English group of Watson, Wilson and Topley[197] infected mice with an animal pathogen, *Salmonella typhimurium*. Webster[198] in America used several animal species and at least 5 infecting organisms. Nutritional deficiencies of the experimental animal were shown to have a strong effect on artificial epidemics. Geneti-

cally determined animal strains of high and low resistance to a particular pathogen and bacterial strains of high and low virulence were tested by variations in diet. Schneider[165b] in extending this work centered on animals of heterogenous random breeding and a bacterial population of similar characteristics, as showing the greatest effect.

Animals with viral infections and a nutritional deficiency may manifest either lowered or increased resistance, the one as often as the other[53]. A lack of discernible effect occurs with equal frequency. Synergism is reported in psittacosis[149]. The opposite effect of antagonism[36] is seen in the increased resistance to Lansing type poliomyelitis virus of mice with thiamin deficiency.

So far as may be determined from review of an extensive literature replete with conflicting and incomplete evidence the generalization follows that where nutritional deficiency is held to have an effect on infection, the expected result with bacteria, rickettsiae[36] and helminths is synergism. Either antagonism or synergism occur with viruses and protozoa, the effect being most evident where the parasite acts intracellularly. What happens then is largely dependent on the relative requirements of the agent for a specific nutrient as compared with those of the host. Because of highly specialized metabolic requirements, and a dependence on specific cellular enzyme systems, viruses are commonly inhibited in pathogenicity by nutritional deficiencies.

NEOPLASTIC DISEASES. The ubiquity of neoplasms in all population groups, their ability to attack most tissues of the body and a relatively long duration are sufficient to account for the frequency with which malignant tumors are found in association with other kinds of disease. An unusual example of synergism in malignancy is the Berry-Dedrick phenomenon[22]. Infectious myxomatosis of rabbits is a rapidly growing sarcoma caused by the virus of benign fibromatosis and a heat stable factor resisting temperatures of 60 to 75°C.

A clear-cut example of antagonism is the destruction of tumors by viruses. Rous[161] observed in 1911, that chickens with virus-induced fowl sarcoma experienced marked regression of tumors with spontaneous appearance of an acute infection characterized by rhinitis and conjunctivitis. The ability of viruses to localize in tumors and interfere with tumor growth has had experimental confirmation[120]. In recent years, oncolytic activity of arthropod-borne encephalitis viruses has had attention[113,138,167]. Some become specifically adapted to types of tumor tissue with enhanced ability to inhibit tumor growth.

Schistosomiasis produces chronic inflammatory reactions of bladder, liver or rectum, subject in later stages to neoplastic degeneration[125]. Pernicious anemia is 6 times as frequent in patients with gastric carcinoma[156] as in other intestinal malignancies and cancer of the stomach 3 times as common in patients with pernicious anemia as in the general population. Whether such associations[195] represent synergistic action of 2 diseases or a relation of cause and effect is indeterminable with etiology in neoplastic disease so poorly understood.

TRAUMATIC INJURY. An association of traumatic injury and infection is seen in the frequent relapse of malaria after operations, fractures or similar experience[27]. Tonsillectomy[11] and immunization procedures[4] have had much recent attention in relation to paralytic poliomyelitis.

Multiple Infectious Agents in a Single Host. Combinations of 2 or more diseases concurrently active in a single host have thus far been limited to situations

where the primary condition was of non-infective nature and the second commonly an infection, although not invariably so. The initial illness usually antedated the second by an appreciable interval. The issue of whether their common presence led to an improved or an exaggerated effect or no change at all was in large measure determined by the existing state of body resistance resulting from the first disease. Host factors were the main consideration. In considering now the association of two infections, an acute process superimposed upon a long enduring and chronic infection presents much the same relationships.

Occurrence of 2 infections, by their nature, brings a more complicated state, through questions of symbiotic or antibiotic action of the 2 infectious agents, a more complex disease through concurrent involvement of tissues previously unaffected, and greater drain on body defenses, specific and innate. The objective in examining such situations is as before, to determine whether the association reacts unfavorably to the host as synergism, or leads to partial neutralization of effect and an antagonism favorable to the host.

HELMINTHS AND PROTOZOA. A complete range from synergism to antagonism is seen in murine bartonellosis. *Haemobartonella muris* causes a severe and fatal hemolytic anemia in splenectomized mice or rats[185b]. Splenectomy also induces non-fatal relapse of a preexisting eperythrozoon infection of mice, another protozoal disease. Either a relapsing natural eperythrozoon infection or one induced by inoculation consistently represses bartonellemia. Mice critically ill with bartonellosis promptly recover, with the disease often reappearing several weeks later as eperythrozoon infection subsides. Hsu and Geiman[94] found that the African strain of rodent malaria, *P. berghei,* caused relapse of latent bartonellosis, an opposite or synergistic effect. In man, malaria superimposed on Oroya fever[146] in similar manner gives rise to a fulminating combined infection.

Synergistic bacterial infection of ulcers caused by *Endameba histolytica* is a common observation in tropical countries. Symptomatic relief in severe amebic dysentery often follows administration of antibiotic or chemotherapeutic agents having no known action on ameba, but presumably affording relief through eliminating secondary infection. The association between amebae and bacteria may be more than simple secondary infection, for amebae in culture[46] depend for growth on provision of nutrients by bacteria. Whether that holds in the human host is not known, but experimental evidence[174] suggests that it does. Cleveland and Sanders[37] produced liver abscess in kittens more consistently through mixtures of amebae and bacteria than by amebae alone. The opposite effect is also seen, with acute bacillary dysentery transforming amebiasis from the asymptomatic carrier state to active dysentery.

A complex synergistic relationship is seen in blackhead disease of turkeys[185a] caused by dual action of cecal worms and histomonads. Ova of the worm, carrying the histomonad, enter the intestinal tract. Larvae develop, burrow into the cecal mucosa and release the histomonads to cause multiple abscesses in the intestinal wall and liver. Ova again transmit the histomonads to new hosts. Histomonads are unable themselves to penetrate the cecal mucosa, and thus are dependent upon the worm for transport between hosts and also within hosts to produce disease. The worm acts as a vector and yet the situation is synergistic, for the worm also produces disease, thus differing from the usual concept of a vector, an arthropod for instance. A similar circumstance has been described in the multiple synergistic effects associated with swine in-

fluenza, which include action of a bacterium, a virus, lung worms, and earth worms[168b].

Antagonism between parasites is less common than synergism, and best evidenced by micro-organisms of intracellular localization. Induced malaria through simultaneous injection of *P. vivax* and *P. falciparum*[28,100] in treatment of general paresis resulted in suppression of *P. vivax* while *P. falciparum* ran a usual course. This demonstrated antagonism has support in field observations. Clark[35] observed that hyperendemic malaria infection in Chagres river villages in Panama was uniformly *P. falciparum*. As the parasite index fell to low levels through chemoprophylaxis, *P. vivax* predominated. During the past war, malaria parasites were demonstrated in the blood of some 1,000 of 3,000 Negro troops in Liberia[7]; only 2 were *P. vivax*, the rest *P. falciparum*. The unit was moved to a non-malarial district in North Africa. Two months later, more than 60 men had active relapse of *P. vivax* infection, undoubtedly acquired at the time of the original *P. falciparum* infection, but there suppressed only to relapse later.

BACTERIA. When a bacterium is associated with another infectious agent or another disease process, the result is likely to be synergistic, whether the association is with a nutritional deficiency, a helminth or protozoon, or when another bacterium is involved. The synergism may be specific, with the reaction limited to 2 particular agents, or it may be non-specific.

The original description of *Clostridium oedematiens* by Novy[142], in 1894, included the observation that virulence was greatly enhanced if mixed with nonpathogenic aerobes. Subsequent experience has extended this relationship to recognition as a principle, that any clostridial infection is more severe in the presence of other bacteria, including other clostridia. In recent military experience[129] fatality from *Cl. welchii* increased from 39% when acting alone, to 66% when other clostridia were present; that of *Cl. septicum* from 42% to 70%; and *Cl. histolyticum* infection, never present alone, was highly fatal. The association of strains producing hyaluronidase with others lacking that capacity was identified[127] as one factor in such differences. *Cl. histolyticum*, for instance, causes intense local tissue destruction in guinea pigs but does not spread; combined with hyaluronidase-producing strains of *Cl. septicum* or *Cl. welchii*, spread is rapid with marked digestion and rapid death. With a strongly hyaluronidase producing strain of streptococcus, death followed even more rapidly. Injection of hyaluronidase did not increase spread of infection in animals with *Cl. histolyticum* or streptococcal infection alone, indicating other mechanisms in synergism than action of hyaluronidase. A synergism of two micro-aerophilic organisms in gangrene of the skin was the basis for the theoretical consideration of bacterial synergism developed by Meleney[134].

Of combinations of 2 acute communicable diseases of bacterial origin that of scarlet fever and diphtheria is outstanding[65b]. Complications of scarlet fever have furthermore been demonstrated as commonly due to a strain different from that causing the original infection[3].

A chance event in the course of studies in experimental epidemiology is one of the few recorded illustrations of bacterial antagonism. Almost all deaths in a colony of mice experimentally infected with pasteurella[71], were due in the course of a year to that organism. *Salmonella typhimurium* infection was accidentally introduced by immigrants to the colony. For the next 6 months most deaths were from salmonella, and none from pasteurella. Eventually one mouse died of combined infection and subsequently pasteurella again was es-

tablished as the principle cause of death, salmonella infection declining to minimal levels. Waksman[193] has summarized antagonistic action of antibiotics in bacterial infections.

BACTERIUM-VIRUS COMBINATIONS. Association of a bacterium with a virus, in agreement with the generalization already made, is almost always cooperative and mutually beneficial. In some pairs the virus lowers host resistance for the bacterium; and in other situations the process is reversed, the bacterium acting on the host to the advantage of the virus. The action commonly involves infections of the respiratory tract at the mucosal barrier.

Pandemic influenza is characterized by common association of pathogens. *H. influenzae* was identified with such regularity[24] in the 1889-1891 epidemic as to be considered the direct causative factor. That experience was repeated in various areas in 1918, sometimes to the extent of 80% to 100% of cases. In other localities, *H. influenzae* was not found, the predominant organism being sometimes a pneumococcus[90], in other instances a hemolytic streptococcus[126] or a green producing streptococcus[133]. The impression still persists from personal experience in that outbreak, of the extent to which a single secondary invader dominated a given situation, exerting an impressive influence on clinical behavior and death rate. With successful demonstration of the viral etiology of the disease, the relationship of the Pfeiffer bacillus and other bacterial pathogens has had less attention or been considered as no more than a coincidence. The existence of other similar associations with accepted interaction of virus and bacterium, scarcely permits discarding this circumstance with either ease[199] or security[168].

The best demonstrated specific synergism is in swine influenza[168a] where concurrent action of a virus and *H. influenzae suis* are required for typical fulminating infection, either element alone being relatively innocuous. The natural distemper of dogs[108] is another, with association of a virus and *B. bronchisepticus*.

The presence of group C streptococci in experimental infection of ferrets with influenza virus[29,63] leads to an altered and more severe disease through nonspecific synergistic action. Other studies[80] show similar relationship between influenza virus and pneumococci in pneumonia of mice. Feline distemper[77] in city populations of cats corresponds to measles among children, with symptoms commonly exaggerated by secondary invaders. The secondary effects of hemolytic streptococci in the great measles epidemics of World War I[38] were such as to remain still vivid to those who saw them. The action of *Salmonella typhimurium* infection in precipitating relapse[137] of latent psittacosis in birds is illustrative of the same general phenomenon. The synergism between bacterial conjunctivitis and trachoma[184b,202] is firmly established, but not as an obligate relationship, for in some parts of the world trachoma consistently develops and progresses without demonstrable secondary infection. In much of Asia and the Middle East[202] trachoma tends to become clinically apparent only in the face of acute bacterial ophthalmia, with Koch-Weeks bacillus or the gonococcus the usual agents. An epidemiological significance rests in more ready transmission of the viral agent of trachoma through increased purulent discharges.

The situations thus far presented have to do with synergism between a pre-existing virus and a subsequently introduced bacterium. Influenzal infection implanted on tuberculosis represents the opposite circumstance, of virus succeeding bacterium. Hawes[81] found opinion in the United States after the pandemic of influenza in 1918 to range from suggestion that tuberculosis

gave protection against influenza to the opposite view that the diseases were synergistic. Rich[155] in reviewing the evidence could come to no definite decision, although convinced that measles as an example of another subsequent viral infection does have a deleterious effect on tuberculosis. Experimentally induced infection with pneumonia-virus of mice or with PR 8 influenza virus[190] causes distinct aggravation of tuberculosis in mice, compared with controls. Chronic dermatoses of an allergic-infectious nature, acne, staphylo-streptoderma, lichen planus, atopic eczema and others, undergo relapse after a variety of virus infections, the so-called virus-pyogen sequence of Stokes[178].

Antagonistic relationships between bacterium and virus are rare. Infection is usually favored by injury to the mucosal barrier of the upper respiratory tract. Armstrong[8] found, however, that experimentally induced bacterial inflammation blocked the usual pathways of spread for subsequently administered virus of St. Louis encephalitis, the bacterium-treated groups being 2 to 3 times as resistant as controls. The effect did not follow if bacteria were installed in the nose along with the virus, or if the test by virus was intracerebral.

Bolin and Anderson[25] found erratic fluctuations in infectivity of mouse-hamster virus given orally to mice, but not after injection into brain or footpad. The explanation was found in contamination of some lots of inoculum with *Micrococcus varians*, a bacterium of itself nonpathogenic and having no effect on the virus in vitro, when given parenterally, or if non-viable.

Virus versus Virus. Virus infections of plants and of bacteria differ from those of animals in many ways, but the main biologic characteristics of the 3 groups are basically similar[18]. Viruses of plants and bacteria have served usefully in defining principles of virus activity, since most of the differences contribute to simpler experimental conditions. Investigation of synergistic and antagonistic relationships is made to advantage at the cellular level and cells of plants and bacteria are well suited to the purpose. The interference phenomenon characteristic of a number of viruses, is the best defined example of antagonism.

PLANT AND BACTERIAL VIRUSES. One difference between plant and bacterial viruses (bacteriophage) is that some plant viruses may act synergistically while known bacterial viruses lack that quality. Synergism in plant viruses is restricted to serologically unrelated species[17], antagonism or interference chiefly to related strains, although all viruses of a serological group are not necessarily antagonistic[20]. An early example of synergism is that between potato X and Y viruses, strains that are serologically and morphologically distinct. X virus is found in many healthy plants; and Y virus, transmitted by aphids, causes a mild disease of potato plants. X and Y viruses in combination produce a severe infection of a plant, in tobacco and potato plants a fivefold increase[158] in amount of X virus over that found in X virus infection alone. Many similar instances are known[151,158], enough to permit the generalization[17] that infection with pairs of unrelated viruses is common, and that symptoms tend to be more severe and of different nature than those resulting from either virus alone. Related strains of plant viruses interfere, a phenomenon first observed in plants by McKinney, in 1929[128], and Thung, in 1931[183]. By contrast, unrelated bacterial viruses typically show complete antagonism[45b] or interference, while related viruses grow simultaneously but not synergistically in cells.

The detailed analyses of interference from studies of bacterial viruses, especially the coliphages[21,124] have greatly aided understanding of animal viruses.

Among defined characteristics of bacterial viruses are a latent period of growth[45b]; the eclipse phenomenon or disappearance of virus as such in the early latent period[47,102]; mutual exclusion[21] of serologically unrelated viruses, only one being able to grow in any one cell; hybridization or re-combination[88] of serologically related viruses with ability to grow in single cells; multiplicity reactivation[124] of partially inactivated viruses; and the "stimulation to breakdown" phenomenon[119] wherein active destruction of closely related viruses occurs.

SYNERGISM OF ANIMAL VIRUSES. Synergism among animal viruses is rare and antagonism common. The first reported instance of viral synergism antedated recognition of the more common interference phenomenon. Gordon *et al.*, in 1932[68], in studies of louping ill of sheep, found that ticks carried the virus of tick-borne fever as well as that of louping ill. Tick-borne fever is an acute innocuous febrile illness of sheep, while louping ill has long been identified as a highly fatal disease of the central nervous system. Experimental infection of sheep with virus of louping ill alone occasionally ended in death but rarely was associated with neural involvement. Only combined infection with the 2 viruses produced severe central nervous system derangement and the characteristic high fatality. A similar situation has been demonstrated[54] for Columbia SK virus and either MEF-1 or Watt mouse encephalomyelitis viruses; for viscerotropic and neurotropic strains of yellow fever viruses[182] when administered intracerebrally; and for herpes and pseudorabies viruses[118] in mice.

NEUTRAL DUAL INFECTIONS BY VIRUSES. Combined viral infections resulting in asynergy are frequent[53]. That these concurrent infections with neutral effect are not necessarily due to invasion of separate cells has been proved by Syverton and Berry[181] and by Anderson[5]. Super-infection of cells with more than one virus showed inclusion bodies of 2 and even 3 viruses in a single cell, with confirmation by animal passage and serological tests.

ANTAGONISM BETWEEN ANIMAL VIRUSES. Antagonism between multiple viruses acting on a single host is known as the interference phenomenon. Early clinical recognition of interference among viral diseases of animals and man is found in observations of Jenner[101] on vaccinia and herpes, and Henle[84] noted a number of such relationships. Priority in experimental demonstration[85] is commonly given to Magrassi[130] and Hoskins[92]. Earlier work included that of Puntoni[152], in 1921, on street and fixed strains of rabies virus, of Gildemeister and Herzberg[60], in 1925, on vaccinia and herpes viruses and of Collier[39] on fowl plague. Magrassi inoculated rabbits with non-encephalitogenic herpes virus and 5 days later gave an encephalitogenic strain intracerebrally without producing symptoms. Hoskins showed that rhesus monkeys were protected against highly lethal viscerotropic yellow fever virus by previous inoculation of a neurotropic strain capable only of producing mild illness when administered peripherally. Findlay and MacCallum[55] demonstrated similar protection of monkeys against yellow fever virus by the completely unrelated virus of Rift Valley fever, and in mice the reverse effect of yellow fever virus protection against Rift Valley fever infection.

Classification of Virus Pairing. Interference between viruses of plant origin depends on their being immunologically related, of bacterial viruses that they are unrelated. Animal viruses follow no such simple division for both related and unrelated viruses may interfere or may co-exist in tissue of a host without demonstrable effect[85]. With no correlation between interference and antigenic

criteria, particle size, or known biological differences, other means of classification are sought. Since interference gives strong suggestion of being dependent on competition between viruses for metabolic functions of a cell, a reasonable basis for classification would appear to be the enzyme systems for which they compete[61a]. An influenza-Newcastle group is thus distinguished from a mumps-PVM (pneumonia virus of mice) group.

Host Factors in Interference. Laboratory demonstration of interference is possible with many mammalian hosts[85] ranging from mice to man, in birds and the chick embryo, and in tissue cultures. Host specificity of the interference phenomenon is sometimes more restricted than the host range of the individual viruses[104,188], for example, interference between lymphocytic choriomeningitis virus and that of poliomyelitis[44a] is definite in monkeys and does not occur in mice.

Interference may take place in a wide range of tissues within a particular host, corresponding to a general infection[163a] or may be limited to selected tissue[116], as of the central nervous system, skin, respiratory tract, or cornea.

Antagonism between viruses is seemingly determined by the interfering virus so modifying the cell that the second cannot develop[87]. Evidence is lacking that viruses themselves have direct effect one on the other. Some viruses stimulate cell growth, best seen in the hyperplasia of virus-induced tumors. Others exert a lethal or cytopathogenic effect, sometimes rapid as with equine encephalitis virus[95a] or slow as with the virus of poliomyelitis[157]. In analogy with bacterial viruses[124], and on the basis of studies on the virus of influenza[141], viruses presumably enter a cell, take over the metabolic functions and redirect the energy of the cell to production of virus. Such virus production may permit or even encourage cellular multiplication, or inhibit it to the extent of killing the cell. Other more complex mechanisms are undoubtedly involved.

Factors of the Agent in Interference. Interference is to a large extent governed and determined by the developmental cycle of viruses and the mechanisms by which viruses multiply. Study of the phenomenon of interference has itself contributed much to existing knowledge, primarily an appreciation that viral multiplication is closely integrated with cellular metabolism and that it is much more complex than the simple binary fission assumed from analogy with bacteria. Profitable investigations, chiefly with an influenza virus-chick embryo system, by Hoyle[93a,b] and by Henle[85] and his associates, have distinguished progressive stages in the development of animal viruses. In order, are an adsorption of virus to host cell receptors, penetration of cell wall and disintegration of the virus, a lag phase or eclipse phenomenon after breakdown of virus into its component parts, replication of precursor fractions, resynthesis, excretion of active virus, and finally a phase of host cell destruction and an end of virus production.

Virus particles of a species, subjected to ultraviolet irradiation[85] or other physical or chemical agents, are deprived of functions in predictable sequence. Influenza virus from chick embryo sources[86a] loses, in order, its infectivity and ability to multiply, then its direct toxic effects, the interfering capacity and ability to inhibit cell growth, and finally adsorbability to host cells and attendant hemagglutination. Partial inactivation serves usefully to simplify experimental procedures and to reduce variables. It has permitted recognition that interference is a capacity of the viral particle itself operating within the cell, and has explained auto-interference[163b], that concentrated inocula

often do not produce as active growth as dilute doses.

Evidence[33] accumulates that animal viruses, like bacterial viruses experience genetic modifications through interexchange of integral viral parts during intracellular development; that is, virus particles break into smaller fractions within the cell and can recombine with fractions of other virus particles that have entered at the same time. The phenomenon is known as recombination, and multiplicity reactivation when applied to partially inactivated viruses.

Exclusion of a second virus in the process of interference may be partial or complete[163a], whether in the living host[55,85] or in tissue culture[117]. Interference is also facultative[179]. By varying experimental conditions[122], the result is sometimes interference and sometimes merely coexistence. Mutual interference, the ability of 2 viruses to interfere with each other in the reverse order, or reciprocally, cannot always be demonstrated[53,116], because of technical considerations, commonly because one virus is so rapidly lethal that the animal or tissue does not survive for test of interference with the less pathogenic agent.

Amount of Virus and Time as Factors in Interference. The number of virus particles that must enter a host cell in order to induce interference is best known for the influenza virus-chick embryo system. Isaacs and Edney[98] came to the conclusion that about 4 virus particles per allantoic cell suffices for interference. The rate at which interference takes place is a function of the speed with which virus reaches and penetrates host cells. The range is from minutes to days, depending upon the host, the virus and the route of injection. Applied directly, as in the chick embryo and in tissue culture, the reaction is usually rapid, and sometimes almost instantaneous.

Duration of interference varies from a few days to several weeks[116]. Reliable limits are possible only with antigenically unrelated viruses, since transition from interference to immunity with related viruses is imperceptible[64]. Good reason exists to postulate interference as long as virus is present in a cell, with the expectation that natural mechanisms of resistance will eventually eliminate the virus. Chronic virus carriers presumably show interference for long periods. Evidence that interference sometimes continues for a time after virus is no longer recoverable is had from a number of observations[154,164b].

Some viruses can interfere with virus already within a host, the time relation depending upon differences in speed with which the two reach susceptible cells. So far as indicated by studies on influenza virus[85] once cellular invasion occurs, intracellular virus can be reached only by homologous interfering viruses up to a prescribed stage in the developmental cycle. Heterologous viruses are apparently not exposed to danger of interference once inside cells.

Mechanism of Interference. Several ingenious theories on the mechanism of the interference phenomenon[44b,85,116,163b] have resolved into the view that 2 concepts warrant serious consideration.

The penetration hypothesis, with several variants[44b] has little experimental support. The preferred interpretation is that interference occurs when one virus takes over metabolic functions of a cell which are essential to the growth of other viruses and thus prevents their development; with the possibility that this is a part of the broader phenomenon of biological antagonism so ably presented by Martin[132]. Interference occurs when a second virus attempts entrance of a cell already occupied by a virus which has monopolized metabolic functions needed by the second virus. The ex-

cluded virus may undergo fragmentation into precursor particles which is the first step of multiplication, but since it cannot synthesize new virus this fragmentation leads to its destruction.

Epidemiological Relationships. Findlay and MacCallum[55] were the first to suggest an influence of the interference phenomenon on epidemiologic distributions of disease. Yellow fever and Rift Valley fever have independent and separate geographic distributions in Africa. The possibility was advanced that Rift Valley fever interferes with yellow fever in nature, and conceivably forms a biologic barrier preventing eastward advances of the yellow fever virus. The 2 infectious agents are known to interfere in experimental animals[55]. The geographic limitations of yellow fever to South and Central America and central and western Africa has long been an intriguing situation. Suitable vectors and susceptible hosts are available in many parts of the world, especially Asia, with many good reasons why yellow fever should be expected to invade a number of tropical and subtropical areas. An hypothesis similar to that of Findlay and MacCallum has been advanced in relation to yellow fever and dengue, the viruses of which are also known to interfere in animals and partially in Aedes mosquitoes[162]. Dengue is prevalent throughout Asia where yellow fever has never been recognized. The vectors of the 2 diseases are the same and both produce long continued infections in Aedes mosquitoes. Interference conceivably is a possible explanation, in human host, animal reservoir or vector.

Similar possibilities are suggested by the limitation in range of eastern and western strains of equine encephalitis in the United States. The geographical separation is so distinct that in only 4 states have both types of the disease been recognized, and these at different times and places[144]. The epidemiological situation is in a manner anomalous, for no significant immunological cross protection can be demonstrated. The 2 viruses do interfere. The vectors of the western strain have been fairly well determined[78], those of the eastern variety less satisfactorily. The reservoirs of both viruses appear to be wild and domestic birds[79], many of which experience an asymptomatic viremia for varying periods as the result of infection. The most likely explanation of these geographical ranges is in some peculiarity of environment or vector but the possibility of mutual interference in animal reservoir or in vector warrants study.

Such evidence as exists on a possible role of the interference phenomenon in temporal relationships of communicable diseases, indicates that either antagonism[176] or synergism may occur in contrast to an effect on geographic patterns where the suggestion thus far is wholly of antagonism. During the 1918 pandemic of influenza[7b], efforts to relate the outbreak to several other epidemic diseases had much attention. The concurrent appearance of pandemic influenza and encephalitis lethargica led to study of data from many sources. The consensus as expressed by the Matheson Encephalitis Commission in 1929[49] was that no direct evidence of synergism existed, but carried the reservation that influenza might predispose to encephalitis in some indirect fashion. Although influenza A and B viruses interfere readily in the laboratory, they are occasionally associated in nature. Kilbourne, Anderson and Horsfall[110] reported a particularly well-documented epidemic in an institution for children in which 24% of cases had both A and B infections.

Relationships to Mass Disease. Since pathogenesis of a morbid process in the individual has so many times aided un-

derstanding of the origin and development of mass disease, the sites and mechanisms of interaction between selected diseases are now examined. The examples are in most instances infections, or with one element an infection. The purpose is to indicate the kinds of pairings that might be expected in mass disease. Attention is then directed to the likely means by which information is to be gained.

From tabulated data similar to those of Tables 1 and 2, but relating to classes of infectious agents, selected items are brought together in Table 3 to give a general scheme of origin and occurrence of synergism and antagonism in the mammalian host. The major divisions are according to site in the body and subdivisions by mechanisms conceivably operating at that site. Specific examples are given under each subheading where enough is known to justify inclusion. Considering infection broadly, pathogenic agents causing extracellular infection tend to be synergistic, while parasites so dependent upon the host as to live only within cells, are for the most part antagonistic to other infectious agents.

TABLE 3.—PATHOGENESIS OF AGENT INTERACTION, WITH EXAMPLES OF DISEASE PAIRS

	Synergism	*Antagonism*
I. *Portal of Entry*		
1. Modification of normal defense mechanisms	Congenital defects and infection[75,82]	Bacterial inflammatory reaction and St Louis encephalitis virus[8]
2. Nutritional support or competition	Intestinal bacteria and amebae[16]	Normal bacterial flora and new invaders[201]
3. Specific interaction in preparation for invasion	Swine influenza virus and *H. influenzae suis*[168a]	
4. Direct transport across mucosal barrier	Cecal worm and histomonad, blackhead disease of turkeys[185a]	
II. *Transport in Tissues*	Gas gangrene[127]	Lymphogranuloma venereum virus and syphilis[121]
III. *Extracellular Tissue Effects*		
1. Nutritional	Deficiencies and infections, amebiasis and tuberculosis[109]	Deficiencies and viral or protozoal infection[36]
2. Circulation	Diabetes, arteriosclerosis and secondary infection[131]	
3. Edema	Influenza virus and pneumococcus, mice[80]	Tissue edema and vaccinia[175]
4. Inflammation	Tuberculosis and other pulmonary infections[155]	Localization of infection by nonspecific inflammatory reaction[135]
5. Fibrosis	Silicosis and tuberculosis[115]	Pneumoconiosis and tuberculosis[56]
6. Body temperature elevation	Fever and herpes simplex[196]	Malaria and syphilis[192]
7. Trauma	Poliomyelitis and tonsillectomy[11]	
8. Hormonal	ACTH, cortisone and infections[112]	
IV. *Intracellular Effects*		
1. Penetration of cell wall		Stimulation to breakdown of bacterial viruses. Blocking of neurotoxic effects of influenza virus[119,192]
2. Intracellular enzyme systems		Interference phenomenon of viruses[85]
3. Direct toxic effects		Oncolytic viruses[128]

PATHOGENESIS OF AGENT INTERACTION. Infectious agents of man may meet and compete outside the human host, rarely in the free living state, commonly in a reservoir host. Vectors of disease also establish mutual relations with infectious agents in the external environment, but other than for those rare instances where the vector also acts as an agent of disease[168b,185a], the associations are not concerned with synergism and antagonism in disease of man. Important epidemiological relationships nevertheless may exist, since such processes may prevent or inhibit transmission of agents to the human host.

POTENTIALITY OF PRACTICAL APPLICATION. Existing knowledge of biological mechanisms in synergism and antagonism of disease is scanty, even for the interference phenomenon. Identification of pairings is many times loose, and quantitative information on the mass effect evidenced by populations is

fragmentary. Few practical uses have grown out of such information as exists.

Clinical and experimental evidence is sufficient to assure with fair certainty that these phenomena of disease interaction are reflected in the behavior of mass disease. The problem at hand is to identify associations of significant proportions and to measure the extent and direction of their effect. The anticipated profit is through additions to practical prevention and control; in extension of knowledge of acquired herd resistance; and to gain an improved understanding of the natural history of mass disease through including this evident ecologic factor. The potentiality in these several directions is of such interest as to warrant setting them down, if for no other reason than to indicate the kinds of information needed and to direct investigation.

Prevention and Control. Principle in prevention and control is simple. Synergism is to be evaded and antagonism encouraged. For many recognized synergistic associations, preventive measures exist for one or other of coexisting conditions. Again principle comes into play, to direct effort by choice to the process which predisposes to the other, but practically to put emphasis on that one where preventive method is best developed. Preventive practice gives numerous examples, one of the best being restriction of tonsillectomy[11] and of certain immunization procedures[4] during frequency of poliomyelitis infection as a means of avoiding paralysis. The need is to search for unrecognized associations through survey of diseases of populations in time and place. Recognition of a situation is requisite to prevention.

The principle of inducing one disease to prevent a second is inherent in variolation[13], the basis of protection against smallpox. The mechanism in vaccinia is cross immunity. Antagonism of viruses, the interference phenomenon, is another possibility[32]. No immediate prospect exists for practical prevention of infection through induced virus interference, although such protection is consistently reproducible in the laboratory[85], interfering vaccines having been used experimentally against equine encephalitis[164a], distemper[70] and ectromelia[6].

Another possibility is to adapt the principles of interference to preparation of immunizing agents through limiting the virulence of viruses intended for immunization by combining them with a benign interfering virus. Sabin[162] prepared a vaccine with strains of dengue and yellow fever viruses. The benign 17D strain of yellow fever virus interfered with the mouse adapted strain of dengue virus sufficiently to eliminate the usual mild toxic manifestations. Immunologic response to both viruses was good.

Laboratory Diagnosis. Among all manifestations of disease interaction, only virus interference has led to a diagnostic test. A method of titration of St. Louis encephalitis virus[95b] based on interference with western equine encephalitis virus, gives results as precise as intracerebral inoculation of mice, although less practicable.

Treatment. That treatment with appropriate chemotherapeutic or antibiotic agents is an effective control measure[41] as well as a means to cure disease is increasingly appreciated. Through shortening the period of communicability, fewer active reservoirs of infection are present in a community at a given time[172]. Among multiple infections the principle finds application in the response of trachoma[184a] to sulfonamides, which appear to shorten materially the period of communicability and contribute substantially to the arrest or cure of the disease. Other examples of therapeutic benefit through

treatment of the more susceptible member of a partnership, but with no demonstrated effect on communicability, include acute amebic dysentery with secondary bacterial infection of intestinal ulcers, gas gangrene infection of multiple origin, and the secondary invaders of viral respiratory infection.

Experimental demonstration of the destruction of neoplasms in laboratory animals by neurotropic viruses[113,138,167], and the effect of a polysaccharide from the Friedländer bacillus[61b] on experimental mumps give some promise of eventual therapeutic use of antagonistic agents. Management of herpes simplex infection with vaccinia virus is a clinical procedure[57], while the classical example of treating one disease with another is malarial therapy of syphilis of the central nervous system.

Epidemiological Investigation. The usual epidemiological appraisal of mass disease of communities is in two general directions. A single disease may be examined in its distributions throughout the world, the local situation being compared with that of neighboring areas and in relation to behavior of the process generally. This is the geographical and historical pathology developed by Hirsch[89]. Alternatively, the local region is catalogued in respect to all existing diseases, as in the modern work of Simmons[171] and his associates or in the German group headed by Zeiss[204]. The procedure in both circumstances is much the same. Factors of the environment which influence distributions and frequencies of disease and injury are sought in the physical characteristics of regions concerned, in the biological environment as provided by other living things than man, and in the social environment, determined by the habits and customs of the populations affected[66]. The biological nature and characteristics of host populations are a second association of variables determining disease incidence. The procedure is essentially an ecologic analysis[65a].

A search for synergistic and antagonistic relationships among mass diseases of human populations is advanced as an additional means toward improved understanding of the fundamental epidemiologic situations found in distributions by time and place. Some diseases are regularly grouped together, others show no such associations. Some show undue clinical severity or unusual frequency in a local situation. Explanation is to be sought in some peculiarity of host, agent or environment. The basis of the epidemiologic method is a search among the many that are known. One factor that commonly receives little attention, is the kind and frequency of diseases other than that being studied. The differences in frequency and behavior of tuberculosis in fractions of populations with and without malaria might approach observed differences for that disease between colored and white populations of temperate climates. In malaria itself there is good evidence[35] that the partial antagonism between plasmodial strains in the human host determines to a considerable extent the geographic range of each.

THE COMMUNITY PROBLEM. As is so usual in medicine, the existence of synergism and antagonism in disease was first recognized through clinical experience with patients. Most of the understanding of these relationships between diseases and between disease and injury, has come through laboratory experiment, primarily experimental infection. The extent to which the presence of one disease in a group of people or in a particular area influences the frequency and severity of others is least well defined, and in fact little studied. Existing evidence is largely the result of incidental observations during other studies, is commonly un-

confirmed and gaps in knowledge are so great as to inhibit development of a reasoned conceptual scheme.

The recorded data of official reports by health agencies are one source of information by which to determine the pattern of occurrence of several diseases in a population unit, and conceivably to obtain some idea of which diseases do and do not occur together. These data serve the first purpose of determining broad population relations, of how frequently one disease exists in relation to others in selected times and in prescribed places. They are not suited to the particular problem of how that fraction of a population with two or more diseases differs from others with a single member of the pair, nor of the relative costs of particular pairings. The single source of information for the United States is in sets of tables listing associated causes of death in three years, 1917, 1925 and 1940[189]. The data were collected for the purpose of studying individual diseases rather than disease associations. Principal causes of death are listed with associated causes as recorded on death certificates. Aside from the recognized errors inherent in such records the main difficulty is inability to derive rates, owing to lack of knowledge of populations at risk. Analysis on a purely comparative basis, through calculating percentage probability of having any one of a number of diseases in association with a single disease, give many irregularities, such as the question of why whooping cough occurs in association with measles so much more frequently than measles occurs in association with whooping cough. The results permit some suggestion of where to look for synergism but not enough to prove a relationship, and the data are wholly unsuited in attempted recognition of antagonism.

Chance Epidemiological Observations. With continuing records of a population 2 diseases might be identified as occurring together in such a way that conclusions as to their synergistic or antagonistic action could be justified. Chance observation also contributes as in the report by Stephens[176] of atypical pneumonia and infectious hepatitis in British troops in Italy during the recent war. A military unit had been experiencing a high incidence of infectious hepatitis. With appearance of a sharp outbreak of atypical pneumonia, Q fever not ruled out, new cases of hepatitis did not occur. The previous high incidence of hepatitis reappeared as the epidemic subsided.

Small Group Studies. Study of small relatively isolated population groups would appear one of the better methods of obtaining epidemiological information on synergism and antagonism, particularly boarding schools and similar institutions. Pickles[147] has demonstrated a tendency for selected outbreaks of common communicable disease to synchronize; Ingalls and Aycock[97] have seen epidemics of acute respiratory infection and poliomyelitis in a school group. Such groupings may indeed represent synergism. For the most part, they are likely to be explained by other and better established factors of host and environment, operating coincidentally to produce two epidemics at the same time.

Experimental Epidemiology. Studies on mouse colonies by the method of experimental epidemiology are well suited to analysis of these complex problems. Observations of Greenwood[71] on the antagonistic effect between Pasteurella and *Salmonella typhimurium* have been described. Based on 2 months in the life of a mouse equalling 5 years for man, Pasteurella infection suppressed *Salmonella typhimurium* for the equivalent of 15 human

years. The obvious question is how common is such an occurrence in other populations and what effect have such phenomena on the genesis and decline of epidemics.

Clinical. Clinicians who approach the patient as a whole, rather than with attention to a specific disease, recognize coexistent morbid processes as constituting a major part of clinical evaluation of a prescribed situation. Individual cases of multiple disease are of principal value in suggesting relationships to be explored in detail in populations of people.

Statistical analysis of hospital records should provide with minimal effort considerable information on disease associations. Adequate controls and large numbers are available in the detailed records of many hospitals and clinics. This approach would seem especially pertinent in geriatrics and in the study of chronic conditions on a population basis. The relationship of hypertension to nutritional disorders, migraine to cerebral hemorrhage, peptic ulcer to liver disease, and chronic infections to malignancy, suggests an imposing list of potential pairs. Of particular interest are mental disorders and personality abnormalities as they relate to organic disease. An outstanding example of this method of clinical study is seen in the collected cases of Joslin[103] for diabetes.

Pathological. A related approach is the analysis of autopsy records, because of ready availability and exactness. Diabetes again provides the example, with demonstration by Warren[195] of high grade association with pancreatic carcinoma as a concrete accomplishment.

Experimental. A principal need is more knowledge of mechanisms by which one disease acts on another. The interference phenomenon would appear well suited to this endeavor. A firm clinical impression is the importance of time relationships. Irrespective of the kinds of pairs involved, much of what happens depends on whether the 2 conditions begin together or one precedes the other; and if onset is separate, which comes first and what is the elapsed interval.

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PROCEEDINGS OF
SESSION OF DECEMBER 16, 1952

The Importance of Metabolic Pathways for the Action of Cardiac Glycosides. SYDNEY ELLIS, M.D., (Dept. of Pharmacology, Temple Univ. School of Medicine). Several reports in the literature indicate that preparations of isolated cardiac muscle do not produce a positive inotropic response when exposed to cardiac glycosides if metabolism has been limited by (a) anoxia, (b) fluoroacetate, which blockades the Krebs cycle, or (c) iodoacetate, which blockades glycolytic energy production. These data are in fundamental disagreement with the hypothesis that the action of cardiac glycosides is upon the metabolic systems involved in the utilization of high-energy phosphate (A. Wollenberger, Pharmacol. Rev., 1, 311, 1949). This problem has been reinvestigated using the Straub preparation of the frog's heart. It is well established that this preparation can maintain satisfactory function under anaerobic conditions only in the presence of a substrate such as glucose, which is able to support anaerobic energy production. Although the anaerobic heart does not respond to strophanthin-k in the absence of extrinsic glucose, this preparation exhibits an excellent positive inotropic response when strophanthin is applied in a medium fortified with glucose. In an analogous fashion fluoroacetate poisoning inhibits the response to cardiac glycosides, but when glucose is supplied to the fluoroacetate-poisoned heart, the response to the glycosides is restored completely to normal. When hearts were poisoned by either iodoacetate or iodoacetamide to the extent that aerobic function was adequate, but anaerobic activity was possible for a few minutes (an indication of mainly glycolytic blockade), such poisoned hearts responded quite normally to strophanthin-k. These results indicate that neither oxidative metabolism nor the Krebs-tricarboxylic acid cycle, nor the glycolytic chain is indispensible for the action of the cardiac glycosides. On the other hand, 2,4-dinitrophenol in low concentrations prevents the action of the cardiac glycosides on the frog's heart (confirming Wollenberger and Karsh, who studied guinea-pig's heart). These data appear to support the hypothesis of Wollenberger in that the systems involved in the production of high-energy phosphate production are not individually essential for the positive inotropic action of the glycosides. The results with dinitrophenol place emphasis on the utilization of high-energy phosphate as the metabolic area in which these drugs act. (This investigation was supported by a research grant H-517 from the National Heart Institute of the National Institute of Health, Public Health Service.)

Studies on Venous Return in Animals with Controlled Cardiac Output. W. J. RASHKIND, M.D., D. H. LEWIS, M.D., J. BOWIE, B.A., D. HEIMAN, A.B. and R. DIETRICK, A.B. (Dept. of Physiology, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania) Studies were made on open-chested dogs with controlled cardiac outputs. The venous return, exclusive of the coronary flow, was diverted into a glass reservoir from which it was

pumped at a constant rate into the right auricle. Arterial and venous pressures, and venous return were recorded simultaneously throughout the experiments. Various procedures were employed, the results of which are summarized in the following table:

Experimental Procedure	*Mean Arterial Pressure*	*Venus Return*
Carotid sinus nerve stimulation	− 18%	−11%
Central vagal stimulation	+ 75%	+ 5%
Injection of commercial epinephrine		
1) 0.2 cc. 1-1000 into rt. auricle	+140%	+ 5%
2) 25 gamma per minute	+ 54%	+ 7%
0.2 cc. 1-1000 l-epinephrine into rt. auricle	+ 70%	+ 5%
0.2 cc. 1-1000 l-nor-epinephrine into rt. auricle	+ 69%	+10%
Opening arterio-venous fistula	− 17%	+10%
Opening arterio-reservoir shunt	− 19%	+25%
Asphyxia phase 1	− 9%	− 5%
phase 2	+ 21%	+12%

Note: All figures expressed as percent change from control. The changes in venous return cannot be explained on the basis of changes in the volume of blood in the thorax, nor on the basis of changes in the volume of blood in the arterial system. It is proposed that these changes are due to active changes in the caliber of postarteriolar vessels, in particular the veins. These data are consistent with the hypothesis of coordinated, unidirectional changes in the tonus of arterioles and venules.

The Refractory Period of Heart Muscle with Special Reference to the Latent Period. J. R. DiPALMA, M.D., (Dept. of Pharmacology, Hahnemann Medical College, Philadelphia). While many measurements of the refractory period of heart muscle have been made, little attention has been paid to the latent period. In this study both modalities were measured with the aid of a dual pulse square wave generator in isolated as well as intact heart muscle of the dog and the cat.

It was found that in normal muscle under standard conditions the refractory and latent periods bear a constant relationship to each other. For example, in isolated atria of the cat it was first possible to cause excitation with a strong stimulus 80 msec. after the onset of contraction (absolute refractory period). However, the extra contraction did not occur until 180 msec. had passed (latent period). Cooling the muscle to 24°C. greatly lengthened the refractory period as evidenced by the depression of excitability during the slow contraction and long relaxation periods but the phenomenon of latency disappeared. It is thus possible at this temperature using strong stimuli to get summation and even incomplete tetanus of heart muscle. Corroborative evidence was obtained in intact dogs and cats. Lowering the body temperature from 30° to 20° C. doubled the P-R and the QRS intervals but the Q-T interval was nearly quadrupled. Despite this great increase in repolarization time it was possible to show consistently that the threshold for tachycardia and fibrillation was lower at the colder temperature. This suggests that the phenomenon of latency may have more relationship to the mechanism of the rapid repetitive arrhythmias of the heart than the refractory period *per se*. Experiments are now in progress to prove this important point more conclusively.

The Effects of Renal Hypertension on Cardiac Dynamics and Cardiac Metabolism in the Dog. J. W. WEST, H. WENDEL, M.D., and E. L. FOLTZ,

M.D. (Departments of Pharmacology and Medicine, University of Pennsylvania). Experimental renal hypertension was induced in normal adult mongrel dogs by Page's method. When persistent hypertension was established, cardiac dynamic and cardiac metabolic determinations were made in the intact state, under morphine and a combination barbiturate anesthesia (dial, urethane, pentobarbital); the Fick method was employed for the determination of cardiac output, and the nitrous oxide method for the determination of the coronary blood flow.

In the hypertensive animal, the average values for the coronary blood flow (cc./100 G/min.) and cardiac oxygen consumption (cc./100 G/min.) were not augmented over the levels for normal dogs. However, since cardiac hypertrophy was a manifestation of this disease, it would seem that the total cardiac oxygen consumption for the augmented work performed would be similar to that of normal dogs under similar levels of work accomplished. Cardiac output was not elevated over the normal values. The mean arterial blood pressure was augmented to an average level of 179 mm. Hg. Hence cardiac work was elevated over the normal levels. Coronary resistance (P/Flow) and total peripheral resistance were increased over the normal values. The cardiac oxygen utilization was similar to that observed in normal dogs.

In the hypertensive preparation a complication which frequently developed was secondary anemia resulting from multiple focal hemorrhages in the gastrointestinal tract, urinary tract, brain, kidney and eye. When no effort was made to restore normal hemoglobin levels, these animals demonstrated higher levels of coronary blood flow and oxygen consumption in comparison with the other hypertensive animals. However, cardiac work and cardiac rate were also somewhat higher than in the non-anemic hypertensive dogs.

Under conditions of these experiments where increased cardiac work was obvious, the total coronary blood flow and cardiac oxygen consumption are not augmented on a unit weight basis of myocardium, but increased only to the extent of the myocardial hypertrophy or greater left ventricular weight. Complicating anemia changes this relationship and would appear to present a limitation on quantitative comparisons of the circulation of normal animals.

BOOK REVIEWS AND NOTICES

SEELISCHE KRISEN IM LEBEN DER FRAU. By PROF. DR. AUGUST MAYER, Tuebingen. Pp. 46. Munich: J. F. Lehmanns Verlag, 1952. Price, DM 3.00.

A consideration of the so-called crises in the life of women. Such include, in certain cases, pubertas precox. Furthermore are treated summarily, the growth of the breasts, the expectation of the first menstruation, the menarche, the psychical effect of overdevelopment or of underdevelopment of the breasts, sexual instinct, the first sexual intercourse, occupation: women's occupation, says the author, is that of wife and mother.

There is a consideration of sexual morality, the present attitude of many women towards sex, extra-marital pregnancy, choice of husbands, suitable ages of the mates, mental crises in healthy marriages, the "in-law" problem, mental crises during the pregnant period, "mixed" (religious) marriages, fear of defective offspring, effect of quarreling during pregnancy, the psychic effect of abortion, fear of labor, twilight sleep, the "bankruptcy of the marriage," the attitude of women toward aging, the difference in this attitude from that of men.

The pamphlet is "multum in parvo." However, the reviewer believes that the attitude of the author toward "modern woman" is somewhat harsh. "Tempora mutantur." Modern woman is the product of changed social, political, and economic conditions, to which she may have contributed wisely or otherwisely. It seems that there will be a long time before purely hygienic considerations will circumvent these conditions, and that there will be a long time before the sociologic pendulum will swing in the opposite direction, if at all. M. M.

WHO ARE THE GUILTY? By DAVID ABRAHAMSEN, M.D. Pp. 340. New York: Rinehart & Co., 1952. Price, $5.00.

THIS is a book written by a man with considerable experience in the field of delinquency. He has chapters on many aspects of the crime problem. He consistently calls attention to the logic of the conclusion that if our present theories are correct and our researches are valid, then the guilty ones in the production of crime are the supposedly healthy and moral sector of the population who are rearing and educating, or failing to educate, our children into socially constructive behavior. He stresses the need of an institution for study and treatment of the delinquent as well as appealing for greater efforts directed toward fostering mental health in the classrooms of our educational system. He makes an eloquent plea for prevention through education. The book is interestingly written, informative, and contains a good bibliography and index, and is heartily recommended for the doctor, the educator, the clergyman, and others interested in the field of delinquency. O. E.

BIOCHEMICAL STUDIES OF BACTERIAL VIRUSES. By E. A. EVANS, JR., Professor and Chairman, Dept. of Biochemistry, Univ. of Chicago. Pp. 68, 24 tables, 6 ills. Chicago: The Univ. of Chicago Press, 1952. Price, $2.75.

A HIGHLY specialized subject, the bacteriophages, is presented as brief lectures. For the medical scientist who is not a virologist, here is an authoritative review of the state of knowledge up to early 1951. There are many who believe that they should know something about bacteriophage research because they have the impression it has produced discoveries of far-reaching significance to medical biology. Such individuals will find this monograph extremely valuable.

Especially to be commended is the author's repeated warning that viruses are too heterogeneous to permit the assumption that mechchanisms operating among bacteriophages can *a priori* be applied to other viruses.

E. S.

RADIOLOGIC DIAGNOSIS OF THE LOWER URINARY TRACT. By DONALD E. BEARD, M.D., WILLIAM E. GOODYEAR, M.D., AND H. STEPHEN WEENS, M.D., Emory University School of Medicine, Atlanta, Ga. Pp. 150; 280 ills. Springfield, Ill.: Charles C Thomas, 1952. Price, $6.50.

THIS book is a valuable aid to both urologist and radiologist because of its complete coverage of all aspects of roentgenology of the lower urinary tract, including the technique of urethrography, anatomy of the parts involved, and interpretation of the urograms. An abundance of roentgenograms of almost every known disease of the lower urinary tract is presented, many of these accompanied by diagrammatic sketches. K. C.